LABORATORY MANUAL
OF
SPOT TESTS

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Foreword

Since the publication of the writer's first comprehensive studies of spot testing and specific reactions such marked progress has been made that this type of analysis and the systematic search for new reagents have become recognized fields of research. The applications of this new approach are gratefully accepted by progressive analysts. This development is also evidenced by the fact that increasing numbers of modern textbooks and laboratory manuals dealing with inorganic analysis are including the use of more and more spot reactions and organic reagents. Consequently, even students in training are now made acquainted with these newer analytical methods.

As to spot analysis in particular, it is now common practice to utilize spot reactions merely as a supplement to qualitative inorganic analysis; therefore, these applications are encountered mostly in the early part of the chemical curriculum. The writer's experience as a college and university teacher leads him to believe that this sort of introduction to spot testing, which usually is not followed by any further use of this technic, is fundamentally wrong. It has little, if any, pedagogic value and bears only the remotest relation to the true province of application of spot analysis. The author has developed, taught, and used spot tests for more than twenty years and so feels competent to assert that under no circumstances should spot test analysis be considered as a mere special technic, to be labelled with the inadequate designation "semi-microanalysis."

Spot analysis admittedly aims at microanalytical objectives. However, it purposefully draws upon and utilizes all sorts of possibilities that may provide usable tests. Consequently, it is linked to so many special fields of chemistry through its theoretical bases that it itself becomes a division of this science. Spot analysis owes its evolution and growth solely to the fact that it was consciously regarded as a branch of "the chemistry of sensitive and unequivocal reactions."

The average student in the early stages of his training is simply not equipped, therefore, to understand the chemical basis of spot reactions, since they involve organic reagents, complex compounds, catalyzed reactions, and so forth. At the most, he acquires a facility that enables him to finish a practice analysis with more dispatch, but he applies these reactions in a purely mechanical fashion. Such blind routine usage can never be a desirable educational goal; the analyst must have a full understanding of all the steps used in the analytical procedure. In other words, he must analyze his method as well as his sample. This is precisely why the classic schemes of qualitative analysis afford a body of instructional material that is so appropriate and also fully adequate for the needs of beginning courses.

The reactions are more uniform with respect to their chemical bases and are more easily understood. When these older methods are conducted on a semimicro scale and as spot reactions, much has been done to further the laudable trend of modern methods of instruction in analytical chemistry: i.e., to teach the student, as early as possible, the need for every economy of time and material.

Spot analysis can be included in the maturer stages of chemical training with entirely different success, and to reach quite different didactic objectives. After the student has completed courses in inorganic, organic, and physical chemistry, he will have the requisite background to understand the use and application of such things as the effect of capillary processes, and will be able to use intelligently organic reagents, complex formation, catalyzed reactions, etc. Furthermore, he will encounter ample opportunity to review and make useful application of many facts that had been treated abstractly in the preceding lecture courses. There are other cogent reasons for postponing spot analysis to a later point in the curriculum. Spot reactions can be used successfully in qualitative organic analysis, in the testing of industrial materials, in studies of rocks and minerals, etc. The immature chemistry student naturally will have no comprehensive appreciation of, or interest in, these particularly excellent applications of spot applications, which may come to have high practical value in his later professional life. This also holds for the use of spot testing in microchemistry. The advanced student, who has already acquired a manual dexterity in the conventional laboratory courses, will be able to master easily the microchemical technic of spot test analysis. He should also be able to decide for himself when and where these methods can be used to advantage. Furthermore, he will be aware of the general limitations that are inherent in microchemical methods and that restrict their employment.

Spot tests are used first of all in the identification of definite materials encountered in the course of the qualitative macro- and microanalysis of inorganic samples. The detection of organic compounds, or of characteristic groups of atoms in such compounds, comes next and leads to the third stage in this progression, namely, the solution of the microchemical problems presented by qualitative inorganic and organic analyses. Spot tests can often furnish the answers to such problems quickly, and with the consumption of very small amounts of materials. Important points can thus be cleared up when testing industrial raw materials and products, in the determination or recognition of rocks and minerals, and in the study of biologically active substances. Spot colorimetry is an excellent example of the possibility of using the new method for quantitative semimicro and micro determinations, which are so valuable as directive or preliminary analyses, or which can be used to advantage in process control.

This extensive and varied field of application of spot testing goes beyond the bounds of a purely analytical objective if the theoretical foundations of the particular methods, together with their relations to the various divisions of chemistry, are kept in mind. A comprehensive survey which thus includes theory as well as practice, will demonstrate clearly the truth of the foregoing statement that spot test analysis constitutes a branch of the chemistry of sensitive and unequivocal reactions. The author is confident that many college and university teachers will concur in his conviction that the aim of chemical instruction includes considerably more than training the student to make a reliable analysis, or to turn out an acceptable preparation, or to take accurate physical measurements. Neither is the learning of isolated facts the sole aim of a chemical education. Extremely important teaching goals are: the closest possible articulation of handiwork and knowledge; the development of a critical sense; the acquisition of the ability to discern the relations of individual observations and findings to each other. Instruction in spot analysis can contribute much to these ends if its manual technic and analytical aims are correlated with the underlying theory. The author, accordingly, believes that there are excellent pedagogic, as well as practical, reasons for including instruction in spot test analysis in the chemical curriculum. The subject should be presented in its total aspects and, consequently, as a special course for adequately prepared students. This book has been written to serve this purpose and to fill a definite need. All parts of the subject were taken into account when the arrangement was planned, and the topics have been treated from viewpoints which the author has found helpful through many years of research, teaching, and actual practice. The professional chemist has also been kept in mind in the planning of this text. It is hoped that he will find here a well rounded and practical presentation of spot test analysis.

The *Manual* opens with a general discussion of the theoretical foundations of the subject. Then follows a chapter on the technic of spot testing, including a description of the necessary equipment. Next comes a chapter, in six parts, giving an extended treatment of surface and capillary effects, which are of such great importance in spot reactions. Appropriate instructive experiments are described. The following chapter deals with spot reactions designed to detect or identify inorganic materials. When planning this chapter, special care was taken to depart from a mere succession of individual tests. Analogies in the chemical bases of the tests were used as the guiding principle in arranging the subject matter. This results in a clear picture of the most varied types of reactions and their analytical utilization. The next chapter, on Qualitative Organic Spot Analyses, consists of three parts: Detection of certain elements in organic compounds; Detection of certain characteristic groups of atoms; Detection of certain

compounds. This arrangement accords best with the fundamentally different types of problems encountered in qualitative organic analysis. The three succeeding chapters deal respectively with the practical application of spot reactions to the testing of: Rocks and minerals; Industrial materials; Biological substances. Numerous practical examples are given in each of these chapters. The *Manual* closes with a chapter on Quantitative Determinations by means of Spot Colorimetry.

The foregoing survey of the text shows that the pedagogic objective has been given a prominent place in all parts of this book. It was for this reason that the chapter dealing with the technic of spot test analysis is developed in such detail, with instructions not only for all the general methods of procedure but also for the installation of a separate laboratory for spot test analysis. A general discussion of the main topics to be treated opens each chapter and division. The chemical basis for every test is given as well as adequate working directions. The choice of experiments has been dictated largely by the desire to use examples which are both characteristic and also of important practical value. Constant stress has been laid on the microchemical aspects. A critical evaluation has been given for each test as to its specificity and selectivity. The interference due to accompanying materials is considered in every case, and directions are given for obviating this interference, provided the "foreign" material does not exceed certain limits. In all, about 180 experiments are included. These can be performed easily in a course extending over 2-3 months, by students who have had some previous training in chemical manipulations. Additional exercises and a more detailed discussion of the underlying theories will be found in the writer's previous books: "Qualitative Analysis by Spot Tests" translated by Janet W. Matthews (second edition, 1939, New York City) and "Specific and Special Reactions" translated by R. E. Oesper (1940, New York City) where pertinent references to the original papers will be found.

Despite the writer's long experience in the field of spot test analysis, he has spent almost two years in composing the manuscript, because he was seeking to give a comprehensive treatment according to correct pedagogic principles in this manual designed especially for teaching purposes. He also had to develop new examples that would be particularly apposite for their educational value. Consequently, this book, in addition to fairly familiar material, contains numerous examples and applications of spot reactions that are published here for the first time. The necessary studies were made in the Laboratorio da Produção Mineral, in which the Ministerio da Agricultura has installed a special research division for spot test analysis and microchemistry.

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TABLE OF CONTENTS

CHAPTER I

THEORETICAL FOUNDATIONS OF SPOT TEST ANALYSIS

It is common practice to determine whether a solution is acid or basic by placing a drop of the liquid on litmus paper, or by bringing a drop of the sample into contact with a drop of indicator solution. In the same way, the completion of certain electrolytic depositions and precipitations can be established by removing a drop of the electrolyte and testing it externally with a drop of an appropriate reagent. Such tests are called "spot reactions." The application and judicious extension of this principle of accomplishing a chemical reaction within the volume of one drop is the basis of the technic variously known as "spot test analysis," "spot tests," "spot analysis," "the spot method," "drop tests." The goal of this branch of qualitative analysis is to develop analogous procedures for the detection or identification of the largest possible number and variety of inorganic and organic materials. A prime characteristic of a true spot test is that it should not require the aid of any optical instrument to determine the outcome of the test.

The substitution of single drops of the test solution and of the reagent, or the use of one drop of the test solution, brought onto a suitable reagent paper, in place of the larger volumes required for test tube reactions, effects a distinct economy of both time and materials. These advantages, in addition to others which will be discussed later, offer cogent arguments for replacing reactions in test tubes, in general, by spot reactions. There were definite reasons why this substitution, beyond a very limited extent, had to be postponed until a rather recent date, but now that the obstacles are overcome, a new technic of qualitative macro- and microanalysis has been developed. Reactions that are satisfactory in test tubes do not always succeed if they are tried by merely mixing drops of the solutions. There are various reasons for this. Certain operations, such as mixing, heating, extraction, etc., cannot be accomplished in the ordinary fashion in spot reactions, and hence demand modifications in working methods. Furthermore, tests are known that are quite good in the usual (2 to 10 ml.) volumes employed in test tube reactions. They fail, however, as spot reactions, not because the concentrations are different, but because the absolute quantity of the characteristic reaction product contained in one drop is not sufficient for it to be positively identified. Consequently, the predominant characteristic of a good spot reaction is that the chemical change must produce a material that can be easily and quickly detected. This is known as the *sensitivity of a reaction.*

Care must always be taken to distinguish between *quantity sensitivity* and *concentration sensitivity*. A precise, numerical expression of quantity sensitivity is given by the *identification limit*. This is defined as the minimum quantity (in micrograms) of material, which can just be revealed by the given reaction.

1 microgram = 1 μg. = 1 gamma (1γ) = 0.001 mg. = 0.000001 g.

If the identification limit of a test is stated to be 0.01 γ the reaction can be regarded as excellent with respect to the quantity of the material that can be detected. However, this figure alone is not sufficient if the over-all sensitivity is being appraised. Obviously it makes a difference whether 0.01 γ of a material can be detected in one micro- or one macro-drop (0.001-0.05 ml.) or in 1, 5, 10 ml., or in a still greater volume. Only when the identification limit is supplemented by a knowledge of the volume in which the detection is just possible, can a statement be made concerning the prevailing dilution. This limiting value of the dilution, which expresses the concentration sensitivity of a test, is known as "the limiting concentration," "dilution limit," or "*concentration limit.*"

The sensitivity of a test (quantity and concentration sensitivity) therefore can be defined or accurately characterized by stating both the identification limit and the concentration limit.

The usual method of determining the sensitivity of a test is to proceed from a solution of known strength. Other solutions are prepared by progressive dilution of this standard solution as long as the test gives positive results. The volume of the diluted solution taken for the test and the weight of the solute contained in it are then known. As soon as these data concerning the identification limit and the volume of the test solution are available, it is easily possible to calculate the corresponding concentration limit. This can be defined as the ratio of the weight (in grams) of the substance to the weight (in grams) of the solution. In the case of aqueous solutions, and particularly if they are dilute, no significant error is introduced by replacing grams of solvent by the volume (in ml.) of the test solution. If the identification limit is expressed in gamma and the volume in ml. then

$$\text{concentration limit} = 1:\frac{\text{volume} \times 10^6}{\text{identification limit}} \qquad (1)$$

For example, if 0.1 γ can be detected in the following volumes of test solution, the corresponding concentration limits are:

$$0.1\gamma \text{ in } 10 \text{ ml.} \cdots 1:\frac{10 \times 10^6}{0.1} = 1:10^8 = 1:100{,}000{,}000$$

$$0.1\gamma \text{ in } 1 \text{ ml.} \cdots 1:\frac{1 \times 10^6}{0.1} = 1:10^7 = 1:10{,}000{,}000$$

$$0.1\gamma \text{ in } 0.05 \text{ ml.} \cdots 1:\frac{0.05 \times 10^6}{0.1} = 1:5 \times 10^6 = 1:500{,}000$$

$$0.1\gamma \text{ in } 0.001 \text{ ml.} \cdots 1:\frac{0.001 \times 10^6}{0.1} = 1:10^4 = 1:10{,}000$$

These sample calculations demonstrate the importance of considering not merely the attainable identification limit but also the respective volumes of the test solutions when appraising the sensitivity of a reaction. The maximum dilution of a solution at which the test still gives positive results can be stated only when this volume is taken into account. The quantity 0.1 γ is, of itself, an extremely small weight of material, and if this minute quantity can be detected in 10 ml. or 1 ml. or even in 0.05 ml., the term "very sensitive" can justifiably be applied to a reaction which succeeds even at these high dilutions. On the other hand, 0.1 γ represents a relatively large quantity of material if it is concentrated within a microdrop (0.001 ml.) where it forms merely a 0.01 per cent solution, which certainly is not a high dilution.

The foregoing calculations demonstrate that the statement of the identification limit alone is not sufficient to judge the sensitivity of a test; the corresponding dilution must also be taken into account. On the other hand, the mere declaration of the dilution is not enough either, for if no more is stated about a test than that it can be carried out successfully at a given dilution, say 1:100,000, no information has been given concerning the volume of this highly diluted solution in which the test is accomplished. Only when this latter figure has also been given is it possible to apply the foregoing formula and calculate the corresponding identification limit from the dilution and volume of the test solution. For example, if a test succeeds in 5 ml. of a solution, whose dilution is 1:100,000, then by equation (1)

$$1:100{,}000 = 1:\frac{5 \times 10^6}{x}$$

$$1:100{,}000 = x:5 \times 10^6$$

$$x = \frac{5 \times 10^6}{10^5} = 50\,\gamma.$$

Consequently, if a test succeeds in 5 ml. of a solution, whose dilution is 1:100,000, and fails if a smaller volume of the test solution is taken, then the corresponding identification limit is 50 γ. A test of this caliber cannot be regarded as sensitive. If, on the contrary, a solution of this same dilution gives a positive result when tried as a spot reaction (0.05 ml.), then the test is really sensitive, as its identification limit is 0.5 γ.

In calculating the dilution limit, only the volume of the test solution *before* the addition of the reagent is used, and not the volume after the

reaction has taken place. The latter volume is sometimes given when a worker is seeking to report the largest possible figure for the dilution. This is completely misleading, because the real and only point at issue is to discover the volume of test solution in which a test is still possible.

The sensitivity of a test must never be judged solely by the smallness of the identification limit (quantity sensitivity) nor by the high dilution (concentration sensitivity) alone; the particular quantity and concentration sensitivity must always be considered conjointly. *Only those tests can be considered sensitive, using the term in its widest sense, which are both quantity- and concentration-sensitive. In other words, the identification limit must be low and the concentration limit high.*

If the sensitivity of a spot reaction is checked by progressively diluting a given standard solution, and then at each dilution, one drop is taken for the test, different observers will practically never agree absolutely in their determinations of the identification limit, even though the same experimental conditions have been closely maintained by all. Almost always there will be a certain range of variation. This variation depends on the individual characteristics of the observers and on whether the eye has been adjusted or trained to perceive slight changes in the reaction system. The range of dilution in which a test sometimes succeeds and sometimes fails is called the "region of uncertain reaction." This region is not peculiar to spot reactions; it is a general characteristic of all types of chemical tests. The region is most extensive in the case of precipitation reactions which give rise to crystalline or amorphous products (supersaturation phenomena, colloid formation, etc.), and less marked in color reactions. Consequently, the statement of the identification limit of a test should always include the quantity of a material agreed upon by unprejudiced observers, who, after frequent repetitions of the procedure, state that this particular quantity can be detected by the formation of a precipitate or the development of a color. In other words, this quantity is definitely outside the region of uncertain reaction.

It must be noted that the identification limit and the concentration limit of an analytical test are not invariable and characteristic quantities, like chemical or physical constants. Entirely apart from the influence of the other materials present, which is often extensive, these figures are always dependent, to a greater or less degree, on the particular volume and the details of the procedure. If, for example, a certain reaction is carried out with a solution of a given concentration (dilution) of the material to be detected and the test is made as a spot reaction, and in volumes of 5 or 50 ml., then the corresponding identification limits and concentration limits will be different in each case. The figures that are valid for a given volume will, as a rule, furnish only an approximate orientation for the values of the

identification limit and concentration limit in volumes that are much larger or considerably smaller. Accordingly, it is not legitimate to calculate these figures for other volumes from data that have been experimentally obtained in some particular volume. An actual verification alone can give validity to such calculated values. It is never proper to state that a reaction has this or that identification limit, but only that a given procedure furnishes this or that identification limit of the reaction in question.

The relation between the sensitivity of a test and the volume of the test solution and the procedure employed is plainly shown by the Prussian blue reaction, which is so often used to detect iron or ferrocyanide ions. The same reaction product, a blue precipitate, is obtained by (1) adding several milliliters of potassium ferrocyanide solution to a ferric chloride solution, and filtering; by bringing together one drop of each of these solutions on (2) a spot plate or (3) on filter paper, or by (4) placing a drop of ferrocyanide solution on filter paper impregnated with ferric chloride. If the identification limit and concentration limit of this Prussian blue test are measured in each of these four procedures, the values are found to be quite discordant. The spot reaction on paper impregnated with ferric chloride gives the most favorable concentration limit for the detection of ferrocyanide, and accordingly will succeed at dilutions where the other procedures have failed.

The reason why the limit of identification and the concentration limit of a test, based on a chemical reaction, depend on the volume of the test solution and on the procedure employed is not necessarily obvious and requires an explanation. A reaction that is used for analytical purposes because it produces slightly soluble or colored soluble compounds is governed by the solubility (ion) product. The relation $[A^+]\,[B^-] = K_{s.p.}$ shows that the solubility of a compound decreases with the value of its solubility product ($K_{s.p.}$). Analogously, a soluble colored compound will be formed with smaller quantities (lower concentrations) of the producing ions A^+ or B^- if the ion-product $[A^+]\,[B^-]$ is small. The visible production of a slightly soluble compound or of a soluble colored material can only result from a chemical reaction if the solubility (ion) product is exceeded. It might be expected therefore that the relation of the concentrations to the solubility (ion) product should also extend to the sensitivity, and consequently the sensitivity of a test should be greater the smaller the solubility (ion) product of the characteristic material whose formation constitutes the basis of the test. However, experience has shown that a low value of the solubility (ion) product is by no means the only factor involved. Not only are precipitation reactions known which prove that compounds with approximately equal solubilities can be detected with different degrees of sensitivity, but cases can be cited in which compounds with higher solubili-

ties are easier to detect than others whose solubility is less. For instance, lead can be detected more easily than cadmium by precipitation as the sulfide, even though the two sulfides have practically the same solubility. Manganous sulfide is more soluble than zinc sulfide, but the precipitation of the former can be detected more readily. Still more striking is the fact that it is easier to detect the formation of lead sulfide than of mercuric sulfide, despite the fact that the latter, with its extremely small solubility product, is by far the least soluble of all the sulfides.

The identification limit of a colored material depends less on the smallness of the particular ion product than on the rapid development and discernibility of a color change. At equal ion products, blues or reds are more easily detected than yellows or browns.

It must be remembered with respect to all precipitation reactions that if the precipitate is to be visible, much more material may have to be thrown down than is necessary merely to exceed a given solubility. There is an apparent contradiction in the fact that a more soluble compound produced by a chemical reaction may be easier to detect than a product whose solubility is lower, and that the ion product is not the sole determinant for the sensitivity of a reaction producing a soluble colored material. The reason for this anomaly is the existence of definite threshold values of particle size and color intensity, that must be reached before a material can be perceived by the human eye. These threshold values are not directly or solely dependent on the slight solubility of a compound, which can be stated numerically, but they depend on additional factors. In precipitation reactions the rate of formation of the precipitate plays an especially important role. The significance of this factor becomes clear if it is remembered that the formal stoichiometric equation representing a chemical reaction presents only the chemical "fate" of the ions or molecules of the solution. The complete picture must include the subsequent aggregation of the individual molecules into larger groups. These go through colloidal states of dispersion, and overcome supersaturation conditions, and prevail over the obstructive influences of the co-solutes on these transformations, which activities do not cease even after formation of the visible precipitate. Freshly precipitated materials often exhibit properties that are quite different from those of aged or ignited materials having the same chemical composition. The explanation of this fact is that even solids can undergo changes such as crystallization or other surface alterations.

The development of a new phase, which is characteristic of a precipitation reaction, is more difficult to detect when colorless, and particularly amorphous, compounds are thrown down than when the precipitate is colored. Consequently, from the standpoint of the analytical chemist, the formation of colored reaction products is especially interesting and desirable.

An instructive demonstration of the significance of color in improving the visibility of a precipitate is furnished by magnesium hydroxide. If the identification limit and concentration limit of the precipitation of $Mg(OH)_2$ by means of an alkali are measured for any method of conducting this reaction, in a test tube or on a spot plate, for instance, the sensitivity will be found to be quite low because small quantities of the colorless precipitate are difficult to see. However, if the precipitation is made in the presence of free iodine or certain dyes, (see p. 104) the iodine or dye is adsorbed on the $Mg(OH)_2$, and even minute quantities, normally not visible, become plainly evident. The effect is so great that the identification limit and the concentration limit of the pure and the adsorptive precipitation of magnesium hydroxide differ by powers of ten. This improvement of the test is solely due to increased visibility of the $Mg(OH)_2$ and not to the formation of a less soluble adsorption compound with a lower solubility product. This is proved by the fact that no magnesium can be detected either by iodine or dye in the alkaline filtrate from a $Mg(OH)_2$ precipitation.

Color reactions, that is the formation of colored precipitates or soluble colored materials, are used so extensively in spot testing that colorless reaction products actually are exceptions in the practical application of this type of analysis. If the drop reactions are conducted by bringing together one drop each of the test solution and the reagent on a porcelain spot plate or on the surface of porous paper, the white background provides excellent contrast for the better perception of small amounts of colored materials. Spot reactions on filter paper are often localized and the products are mostly fixed on the surface of the paper. This obviously is an additional advantage because the solvent then diffuses away through the capillaries of the paper, and there results a quasi-precipitation and filtration in the surface of the paper. The special advantage of using reagent papers in spot test analysis is excellently exemplified by the Prussian blue test on ferric chloride paper (see page 5). The adsorption and localized fixation of the reaction products in the surface of the paper invariably give more favorable values for the identification limit and concentration limit than are obtained by the classical procedure of mixing the test solution and reagent in a test tube. Spot tests on paper are also superior to those made on non-porous substrates, such as spot plates. Certain components of a solution can often be fixed by precipitation on reagent paper, while the non-reacting constituents diffuse away with the water, accumulate around the spot in concentric rings, and can be detected there by means of suitable reagents. There is obviously little need of stressing the advantages of such a simple method of detecting two materials in one drop of a test solution. Capillary and surface reactions are frequently utilized in the conduct of spot tests to improve the discernibility of the results of color reactions.

Spot test analysis is constantly concerned with the effort to develop procedures by which it is possible to detect minimal quantities of materials in one drop. This is the goal of all modifications in the methods of executing reactions that are useful to the analyst. Consequently, spot testing is a division of qualitative microanalysis, whose province is the identification or detection of materials in quantities that are minute, both absolutely or relatively in comparison to the quantity of solvent.

It is entirely proper to judge the utility of a chemical reaction for the microchemical detection of materials solely on the basis of its limit of identification, as the attainable concentration limit may be taken above all as a reliable criterion. *No reaction, or rather no procedure for carrying out a reaction, may be considered suitable for microchemical tests if its identification limit exceeds 10 γ.* This weight is beyond the limits of ordinary determination on an analytical macrobalance. If the identification limit is taken as the criterion of the microchemical utility of a test, this limit becomes of fundamental importance because, as has been pointed out, the identification limit does not refer to the particular volume of the test solution taken, nor to the volume in which the reaction is accomplished. In other words, a test may be microchemical without necessarily employing any of the special microtechnic that is required for the manipulation of small quantities of materials. In the majority of cases, classical qualitative microanalysis has employed the "crystalloscopic" method. In this procedure a crystalline precipitate is produced in a microdrop (0.001 ml.) of the test solution, and the precipitate is examined under the microscope to determine the crystal form. Since these forms are typical of certain compounds, the central point in the procedure is the production of well defined crystals that can be readily identified. This technic is no longer the sole method of conducting a qualitative microanalysis. *The most important feature of the microchemical detection of materials is not the procedure, which is secondary; the dominant factor is the ability to detect minimal quantities.* Consequently, the method of precipitating characteristic crystals should be critically and unhesitatingly compared with those spot reactions which furnish identification limits of the same order of excellence and that can be carried out in macrodrops (0.05 ml.) without recourse to a microscope. There are indeed numerous instances in which spot reactions surpass crystal precipitations in regard to both the absolute and relative quantities that can be detected. An excellent illustration is furnished by two methods of detecting aluminum. The microchemical test, in which a crystalline precipitate of alum is prepared, has an identification limit of 0.35 γ Al. The spot test, in which violet aluminum alizarinate is formed by treating a drop of the test solution with a drop of ammoniacal alizarin solution, has an identification limit of 0.15 γ Al. Consequently, the spot

reaction excels the older procedure not only with respect to quantity sensitivity (identification limit), but the superiority also extends to the concentration sensitivity (concentration limit). If the respective volumes of the test solution, namely 0.001 ml. and 0.05 ml., are used in calculating the corresponding concentration limits by means of equation (1) on page 2, the values will be found to be 1:2900 (alum) and 1:330,000 (alizarinate).

It is not always necessary to use macrodrops of 0.05 ml. for spot tests; frequently microdrops serve equally well. In such cases, even though the concentration limits are not changed much, the identification limits will be still lower. The available quantity of the specimen will determine whether micro or macrodrops should be taken for the test, and also the number of times the test should be repeated. At least one repetition is advisable in every instance.

It is easy to decide when tests that are quantity sensitive should be used for microanalytical purposes or when those that are concentration sensitive should be employed. The former, precipitation of crystals, for instance, are advantageous if only a small quantity of sample is available, and if there are urgent reasons to employ micromanipulations. Typical cases are the detection of possible inclusions and inhomogeneities in technical products, minerals, and so forth. Tests that are concentration sensitive are particularly necessary, and sometimes are indispensable, if the analytical problem is to detect traces of certain materials at high dilutions or in association with large amounts of other materials. These conditions are often encountered in the important tasks of determining the purity of materials and of testing biological specimens. Emich has aptly designated this branch of analytical practice "the search for traces" or "trace detection." In general, the main emphasis will be on concentration sensitive tests. They alone make it possible to apply successfully spot reactions or even test tube reactions at the high dilutions prevailing when minimal quantities of the specimen are put into solution.

It has been stated (page 8) that the maximum value of the identification limit of an acceptable microchemical test is 10 γ. From the standpoint of the microchemist, the detection of this relatively large weight of material can be considered satisfactory only if this quantity is contained in a rather large volume and not, for instance, in a macro or microdrop. The formula given on page 2 shows that the detection of 10 γ in 0.05 ml. corresponds to a concentration limit of 1:5000, whereas the corresponding figure for 10 γ in 10 ml. is 1:100,000. As a rule, the identification limits of microchemical tests employing crystalline precipitates or spot reactions are fractions of a microgram (γ); in isolated instances quantities as low as thousandths of a microgram (milli-microgram = mγ) can be detected. Tests for certain organic compounds by spot reactions are an exception to

these standards. Reactions whose identification limits are as great as several micrograms may be used in these cases, either because more sensitive tests are not known, or because, as is sometimes the case, complicated reactions which do not go to completion must precede the application of the actual spot test.

The identification limits of spot tests uniformly represent minute quantities if expressed in terms of weight. If, however, these weights are translated into the number of molecules that can be detected by sensitive spot reactions, the figures are enormous, because the actual weight of the individual molecules and the space they occupy are so exceedingly small. It is easy to convert identification limits into terms of the corresponding *numbers of molecules*, by using the Avogadro relationship, namely that one gram molecule of any material, no matter what its state of aggregation, contains 6.06×10^{23} individual molecules. If, for instance, 0.005 γ of ammonia is detected by a very sensitive spot reaction, the corresponding number of molecules can be calculated:

17 g. NH_3 contains 6.06×10^{23} molecules

1 g. " " $\frac{6.06 \times 10^{23}}{17}$ "

1 γ " " $\frac{6.06 \times 10^{23}}{17 \times 10^6}$ "

0.005 γ " " $\frac{6.06 \times 10^{23} \times 5}{17 \times 10^6 \times 10^3} = \frac{30.3 \times 10^{14}}{17} = 1.7 \times 10^{14}$ molecules of NH_3.

The foregoing conversion shows that the chemical detection of 0.005 γ of ammonia in one drop is possible only if the enormous number of 170 million million molecules of ammonia are present. *Such startingly large figures are always obtained even when the most sensitive chemical tests are reduced to numbers of molecules.* This finding is due to the fact, stated on page 6, that the perception of colors and solid particles, as well as odors and tastes, by the human sense organs, is limited by threshold values. When observing precipitation reactions it must be remembered that the precipitate as seen does not consist of individual molecules as expressed by the usual stoichiometric equations. The primary formation of the individual molecules of the reaction product is followed by the aggregation and orientation of billions of individual molecules into amorphous solids or crystalline precipitates. Individual molecules exist only in the gaseous state or in solutions. But even in solutions a tremendous number of colored molecules or colored ions are necessary before the eye can perceive a color or a color change.

Consequently, the detection of materials through the optical perception of chemical reaction products presents a lower limit beyond which the hu-

man sight does not function. If the identification limits of chemical tests were lower by several powers of ten, or if the eye could observe much smaller quantities of material, probably it would be found that inorganic compounds are really pure only in very rare cases, and that many contaminants are always present. The term "chemically pure" is, therefore, only relative and refers to a certain degree of purity. Accordingly, statements of purity should be limited to the declaration that certain contaminants could not be detected below a given percentage by the particular analytical procedure employed.

The sensitivities (identification limit and concentration limit) of microchemical tests utilizing crystalline precipitations and spot reactions are adequate for many practical problems presented to the qualitative analyst. Activity can be expected only above a certain quantity of material or a definite dilution range in both inanimate and animate systems, because the effect always proceeds from molecular or ionic species. The detection of extremely small quantities is required particularly often in biological problems; in other cases only if the supply of the specimen is exceedingly small.

It is always important to keep in mind the limits of the applicability of spot test analysis. *With normal chemical reactions this technic can be used to identify the color and aggregation form of reaction products only within the range of physiological perception.* Consequently, highly visible reaction products are of prime importance in spot testing, and all research in the field of spot test analysis is directed toward the discovery of sensitive reactions and the development of means to make the reaction products as visible as possible. Colored compounds are much easier to see than colorless materials, particularly at high dilutions or when finely dispersed. Accordingly, the choice and study of chemical reactions with a view to possible use in spot testing lays especial emphasis on the production of colored compounds. Organic reagents that can react with inorganic compounds to produce colored products thus become particularly important. The search for new organic reagents is justified sufficiently by reason of their reaction sensitivity, but there are additional reasons for favoring this class of reagents.

The analytical value of a chemical reaction is not determined solely by its sensitivity. A factor of equal importance is the certainty of the test, that is, the ability to provide a direct and sure identification in the presence of considerable quantities of other materials, without the necessity of intermediate separations that frequently involve unavoidable losses. The analyst is seldom confronted with an isolated material; usually several others are present, and the problem is to obtain definite tests in mixtures or in a solution containing several solutes. It would be ideal if a suitable test, which would succeed even in the presence of other materials, existed

for every element or for every characteristic inorganic radical or organic group. Such tests would be termed *specific* and the appropriate reagent a *specific reagent.* Tests that are not characteristic for a single material, but that can be applied under the same conditions for several materials, are known as *selective,* and the corresponding reagents are *selective reagents.* These exercise a certain selection from among the large number of materials being tested.

A test (reagent), therefore, is specific, or more or less selective, depending on whether only one material or a greater or smaller number of materials can be characterized by its aid. At present only a few specific reactions and specific reagents are available, but a considerable number of selective tests and selective reagents are known. The dearth of completely unmistakable test reactions, and the many possibilities for variation in the composition of natural and artificial products, are the reasons why qualitative organic and inorganic analyses still require many separations into groups, whose individual members can be detected by characteristic reactions only after such group separations have been made. Nevertheless, impressive progress in the methodology of analysis has been made in the past twenty years through the use of specific and special reactions. This is particularly true for the solution of uncommon analytical problems, which is incomparably easier now because of the availability of more sensitive and more specific reagents, particularly organic ones. Indeed, in many cases, it has been possible to solve such special problems only because new reagents have become available.

The reaction medium also is always involved in the specific or selective action of a reagent and, consequently, attention must be paid to this factor. The maintenance of the proper acidity or alkalinity (adjustment to a definite pH), the conversion of the element to be detected to a certain valence (state of oxidation), and the observance of definite temperature ranges and so forth are important. In inorganic analysis it is sometimes possible to avoid the interference due to the presence of ions that react analogously to the material being sought by converting them into complex ions, whose reactions are of a different nature. This conversion into complex ions, which occasionally raises the selectivity of a reagent to specificity, is called *masking.* It can be accomplished by adding *masking agents,* which are soluble inorganic or organic compounds forming complexes.

Masking reactions, therefore, are chemical changes which occur in aqueous solution and lead to soluble complex compounds. Like all chemical changes, they conform to the law of mass action and chemical equilibrium. Consequently, an ion that forms a complex ion with a masking agent never disappears completely. The measure of the masking is determined by the position of the equilibrium of the masking reaction or, what amounts to the

same thing, by the stability of the complex compound formed. If a complex compound is given the general formula AM, in which A and M are ions, or A is an ion and M a neutral molecule, then

$$AM \rightleftarrows A + M.$$

This equilibrium shows that the original ion A can be liberated and made accessible to its normal reactions by removing the masking agent M from a masked system, which itself is an equilibrium system between the materials participating in the masking. This process is known as *demasking*. A hindered reaction can be reinstated by demasking the system and this release can be utilized to detect a masking or a demasking agent. Both of these procedures are of importance in spot test analysis.

Masking and demasking, accordingly, are analytical utilizations of the formation or the decomposition of soluble complex compounds. In this connection it should also be noted that the result of incorporating an ion or a molecule in a complex compound is not inevitably the more or less extensive masking of the normal reactivity. The opposite can also occur, namely, the enhancement of reactivity by complex binding. For instance, the MoO_4^{--} ion is a weak oxidizing agent, but when incorporated in the complex phosphomolybdic acid its oxidizing power is greater. This observation can be used for the detection of phosphate and silica. The masking and demasking of reactions and the augmentation of reactivity by complex binding indicate the importance of the chemistry of complex compounds in solving analytical problems and, especially, in furthering the aims of spot test analysis.

The reaction medium is of importance in determing not only the specificity or selectivity of tests (reagents) but also the attainable sensitivity. It must be noted that materials which apparently are completely indifferent, because they do not react with the reagents employed, nevertheless can frequently decrease (and to varying degrees) the sensitivity of a test. The influence of "indifferent accompanying materials" depends on their nature and especially on their quantity. They make themselves particularly evident in the range of the limiting dilution. It is often found that the values of the identification limit and concentration limit of a test performed in pure solutions of a material do not agree with those obtained in solutions which also contain other solutes. Thus, strictly speaking, *an absolute certainty of tests is seldom achieved, but rather only a certainty within particular limits of concentrations of accompanying materials.* It is expedient to determine the sensitivity of specific and selective tests not only in pure solutions, but also to establish the values of the identification and concentration limits when the test is performed in the presence of known quantities of indifferent accompanying materials, whose quantities should

be as great as possible. Schoorl has proposed the term "limiting proportion" for the ratio of the quantity of material that can just be determined to that of the accompanying substances. Obviously, all limiting proportions cannot be determined because of the tremendous number of possible variations. In general, only the limiting ratios for those mixtures which are of particular practical interest are stated, such as cobalt in the presence of nickel, iron in the presence of aluminum, and so forth.

It is not possible at present to give a definite, uniform explanation for the lowering of the sensitivity of tests by indifferent materials, since various factors may be involved. In precipitation reactions the effect is probably due to supersaturation and colloidal phenomena which lead to a retardation in the aggregation of particles. Changes in the properties of the solvent are probably the principal factor in the case of color reactions. The specificity and selectivity are impaired by unfavorable limiting ratios, especially in dilute solutions of the materials being detected. This is a further reason for using reactions of the highest possible sensitivity as spot tests. They still permit the attainment of identification limits meeting the requirements of microanalysis, despite unavoidable lowering of the sensitivity because of accompanying materials which are occasionally encountered.

It has been found that sensitive reactions that are also specific or selective can be secured far oftener with organic than with inorganic reagents. Furthermore, definite groups in the organic compounds have been discovered possessing specific or selective action.

This fact is of great significance because the discovery of new organic reagents is no longer the result of hit or miss trials. The great reservoir of organic compounds can be subjected to a critical survey, and possibly definite rules and uniform modes of behavior can be discovered. A certain degree of orientation is provided by the fact that organic reagents are not uniform with regard to their modes of behavior and the composition of their reaction products. Six different types can be distinguished:

(1) Reagents producing normal salts with inorganic cations or anions because of their acid or basic character;
(2) Reagents of acid character capable of producing inner complex salts because of the presence and spatial proximity of atoms capable of coordination through the development of auxiliary valences;
(3) Reagents containing atoms or groups of atoms that can be coordinated, and consequently whose entire molecule can combine with inorganic compounds to form molecular (addition) compounds;
(4) Reagents forming adsorption complexes with inorganic compounds;
(5) Reagents undergoing characteristic color changes on oxidation or reduction; and

(6) Reagents reacting in a characteristic manner after incorporation of inorganic groups.

The activity of certain groups in organic reagents is not reflected solely in the composition and the properties of the respective reaction products with inorganic compounds. Some groups also exert a determining influence on the solubility of organic reagents in water or in organic solvents. This is of importance because tests, for the most part, are made in aqueous solution and, therefore, it is always desirable to have the reagents soluble in water. With a knowledge of the activity of certain groups it is then possible to introduce these into organic compounds and so arrive at improved or new organic reagents by synthetic measures.

The study of the relation of groups in organic compounds to specific and selective action has become a special field of research in analytical chemistry. This cannot be discussed here in detail. However, it can be pointed out that the rules and concepts of the chemistry of complexes play as important a rôle in the search for new reagents as they did in the intelligent application of masking and demasking reactions, so that it is entirely proper to speak of applied complex chemistry. The results of this type of research are of great significance for the whole field of analytical chemistry, and the discovery of new organic reagents of greater sensitivity and selectivity has contributed decisively to the development of spot test analysis.

The selective or specific action of groups in organic compounds toward inorganic ions or molecules makes it possible not only to detect certain inorganic compounds but, *vice versa*, inorganic reagents may be utilized to identify certain groups in organic compounds. This utilization of the relation between groups of atoms and characteristic reactivity was the starting point for the *application of spot reactions in qualitative organic analysis*. It has since been found that many operations of a preparative nature can be carried out on a microscale with organic compounds. These include building up and degradation reactions, oxidations, reductions, and so forth, which lead to the development of groups exhibiting characteristic reactions, and which therefore lend themselves to typical spot tests. The identification limits that can be attained in qualitative organic analysis by means of spot reactions frequently are adequate for microanalysis. However, the detection of organic compounds is often less reliable than the identification of inorganic materials. The reason is that the possibility of forming compounds with organic groups, and their reactivity, is often extensively influenced by the presence of other groups. It is also necessary to note that materials of different constitution may react in different ways with the same reagent. Consequently, organic tests demand particularly a knowledge and consideration of the mechanism underlying the reaction at hand. Frequently the occurrence of certain reactions, especially in

mixtures, can be considered as no more than an orientation, unless other tests have given positive results and thus confirm the finding.

The highest possible sensitivity and the most extensive certainty are the factors of prime importance in spot reactions, rather than the use of old familiar reagents and the biased adherence to a definite technic. The recognition of this situation has resulted in the use in spot test analysis of reactions or effects that have long received little or no attention in classical qualitative analysis. The significance of the chemistry of complex compounds has been indicated in the foregoing discussion of masking and demasking reactions and of organic reagents. Complex reactions of the greatest variety had been used in classical analytical chemistry and spot test analysis was thus able to use, to some extent, these previous observations and also to take advantage of any new discoveries. However, many experiments designed to test and apply new complex reactions originated in work on spot test analysis. An entirely new departure, however, is the *analytical utilization of catalyzed and induced reactions,* which for a long time were not consciously applied in analytical procedures. The great microchemical significance of these types of reactions was established for the first time by studies dealing with spot tests.

Catalysts are materials that raise or lower the rate of chemical reactions. This effect is often accomplished by minimal quantities and *apparently* without direct participation in the reaction. *Such activity of minute quantities of material* is highly desirable for microanalytical purposes. It is known also that the catalytic influences of materials are limited to quite definite reaction systems, in other words, the action is specific. *Consequently, if a catalyst can be identified by means of a catalyzed reaction, the requirements as to sensitivity and certainty will have been met.* It has been found that a whole series of materials can be detected more sensitively and surely by catalytic effects than by other reactions. In practice, when catalyzed reactions are used, either the accelerated formation of a reaction product or the accelerated disappearance of a reactant is noted. No example is known, as yet, of the analytical use of a negative catalyst, or of the poisoning of a positive catalyst. Most of the effects that have been used heretofore are catalyses in homogeneous systems, and deal with reactions of dissolved materials. In some cases, the formation of characteristic reaction products, or the disappearance of reactants can be observed directly. In other instances, this must be evidenced by subsequent reactions, which can be accomplished either during or following the completion of the catalyzed reaction. Sometimes it is best to compare the course of a reaction with and without the catalyst.

Induced reactions are related to those catalyzed reactions in which the catalyst takes an active part through the formation of labile and more

active compounds. In induced reactions, a change which by itself proceeds very slowly is speeded up if a rapid reaction occurs along with it. The reaction which proceeds of itself is the primary, or inducing reaction, the other is the secondary, or induced reaction. As a rule, the primary and secondary reactions have one participant in common, the actor. The diversity in the mechanism of a catalyzed and of an induced reaction lies in the different way in which the actor and the catalyst function. The actor is practically all consumed in the primary reaction; the catalyst forms an active intermediate compound and is regenerated by a secondary reaction. Frequently, there is no external difference between catalyzed and induced reactions, particularly if small quantities of an actor, in other words, an insignificant inducing reaction, suffices to bring about a large induced action.

The attainment of the highest possible sensitivity and certainty of tests leads the advocates of spot test analysis to be interested in the utilization not only of all of the possibilities offered by chemical reactions but also in the direct or supplementary *employment of physical effects*. These include, first of all, *surface* and *capillary* processes which occur when spot reactions are made on paper. The use of filter paper as the substrate for spot reactions not only improves the visibility of colored reaction products through the contrast with the white surroundings, but in addition local accumulations of material may occur as the result of adsorption and diffusion. Sometimes this accumulation accomplishes a capillary separation of the components of the solution, whose presence in different zones can then be established directly or by subsequently applying suitable reagents. The use of reagent papers impregnated with insoluble or slightly soluble reagents is particularly important with reference to the local accumulation of materials. Such papers act like soluble reagents because of the fine state of division of the solid reagent in the capillaries of the paper, but have the advantage that the reaction products remain at their place of formation and thus can be seen directly, or can be detected by after-treatment with suitable reagents. Certain chemical changes can be detected much better if carried out on paper than on non-porous substrates or in test tubes. There are, indeed, certain topochemical effects that can only be made evident if the reactions occur on filter paper. Consequently, these tests can be used for analytical purposes only under these conditions (see Chapter III, p. 63).

In modern chemical analysis, filter paper for separating solid and liquid phases is often replaced by other porous filtering media, such as sintered glass or Gooch crucibles; sometimes the solid is collected and isolated by centrifuging. However, filter paper is such an absolute necessity for certain types of spot tests that it is no longer a mere aid but rather can

be regarded as an active participant in the reaction. The paper, of course, does not appear in the equation representing the reaction, but its indispensability appears in the form of a typical reaction (spot) pictures.

Capillary and surface processes can be utilized for purposes of spot testing in other ways than on or in paper. The *surface tension* at the interface of two immiscible liquids can also cause a local accumulation of slightly soluble reaction products. Consequently, extraction with ether makes it possible to recognize clearly very small quantities of precipitate, which otherwise are scarcely visible. The solid forms a film at the boundary of the water-ether layer. This is a kind of micro-flotation, whose practical application is particularly possible after precipitations from acid solutions. The flotation is not limited to ether; other organic solvents, such as carbon disulfide and carbon tetrachloride, behave similarly.

The surface effects useful for spot test analysis also include the *adsorption of dissolved materials on the free surface of solid adsorbents suspended in water.* Traces of dissolved materials can sometimes be fixed by shaking with suitable adsorbents, and subsequently detected on the adsorbent by spot reactions, after removing the solvent. This accumulation of material can be applied successfully in *trace detection*; the adsorbent then serves as the *trace catcher.* Only a few milligrams of this catcher are necessary entirely to remove small quantities of a material from a large volume (10 to 500 ml.). For example, traces of silver can be adsorbed from aqueous solution by silica gel, and traces of copper by talc or calcium fluoride, and then detected on the adsorbent by sensitive spot reactions. If this method of accumulating a material is to be of analytical value, a selective and extensive adsorption must occur, in which the chemical nature of the adsorbent is also a factor.

The removal of a material dissolved or suspended in water by *extraction* with ether or another immiscible organic solvent can also be looked upon as a physical effect, since it is a phenomenon concerned with the accumulation of material. In this extraction, however, the formation of solvates (complexes between molecules of solute and solvent) plays a great part, so that the process, in principle, is likewise a chemical process. Extraction is of particular value in spot test analysis when there is the possibility of concentrating in a small volume of the extractant the material that originally was distributed throughout a much larger volume. A significant improvement is often obtained thus; for instance, if the colored material can be concentrated by extraction. If an extraction is quantitative, it can be used to isolate particular materials, which can then be detected by special reactions after the extractant has been evaporated.

Magnification by a lens or microscope plays a minor rôle among the applications of physical effects and devices used in spot test analysis. This is

in contrast to that branch of qualitative microanalysis, in which the precipitation of crystalline materials is employed extensively because the definite identification of crystal forms can only be accomplished with the aid of the microscope. Magnification seldom offers any important advantage in observing the color reactions which constitute so great a proportion of the tests used in spot reactions. The use of a magnifying glass is recommended only when observing the local formation of colored reaction products when seeking inhomogeneities or inclusions. This can be done by placing a drop of reagent solution on powders or on sections of solid specimens. The treatment of thin sections with suitable reagents and observation of the reaction picture under the microscope is often of great advantage in mineralogical and metallographic studies. However, this branch of testing material lies outside the field of normal spot test analysis. It is worthy of note, however, that many sensitive reagents that are now successfully used for such purposes were originally employed in spot testing.

The use of the quartz lamp in spot test analysis is of considerable importance. In *fluorescence analysis*, specimens of various kinds are irradiated with ultraviolet light. It is found that certain materials fluoresce or change their color in a characteristic way. In general, very minute quantities of fluorescent materials are sufficient to show this effect. Among purely inorganic compounds, only a few exhibit a characteristic fluorescence. These include many uranium salts, cadmium sulfide, mercurous chloride, and salts of rare earths. It seems that metal and metalloid compounds exhibiting characteristic fluorescence are to be sought chiefly among normal and complex salts that have organic components. On the other hand, fluorescence in ultraviolet light is rather common among purely organic compounds. This phenomenon can be of the highest value in deciding questions regarding the origin of specimens, the presence of impurities, and so forth. When testing organic preparations it is best to dissolve them and place drops of the solution on paper. When these dry, the constituents frequently separate into zones because of adsorption and capillary action, and then fluoresce characteristically when placed under the quartz lamp (drop capillary picture). Until recently the fluorescence analysis of inorganic and organic compounds has consisted almost exclusively of testing the fluorescence of finished products after the addition of necessary acids, bases, or salts. Spot test analysis has lately opened a promising new field based on the *synthesis of fluorescent compounds*. Certain fluorescent compounds can be prepared by quite definite reactions, and in these, as a rule, the fluorescence is due to the formation of certain groups or special bondings. Consequently, if an unknown material is subjected to a reaction of this kind and a characteristic fluorescence results, the presence of a particu-

lar starting material is definitely indicated. On the other hand, if the change does not result in a fluorescence, the conclusion is that certain original materials were absent.

In fluorescence analysis and its application to spot tests not only the appearance or the characteristic change in fluorescence is to be noted, but also the disappearance (extinction) of fluorescence by chemical action. If this extinction is a typical effect of particular substances, then there arises the possibility of an analytical application.

The use of the quartz lamp in spot tests is not confined to the demonstration of fluorescence. The occurrence of photochemical processes likewise can often be quickly and easily established by means of drop reactions on paper. Sometimes these can be used as sensitive tests. Photochemical reactions are chemical changes or decompositions that are accelerated or initiated by light of short wave lengths.

Adequate quantities of a particular species of molecule or ion must be involved in reactions that are of real value to the analyst. It is not absolutely necessary to prepare a test solution to carry out drop reactions. Reliable results can often be achieved by merely putting a drop of the reagent on the solid specimen, or by pressing reagent paper on the specimen. In the latter case negative prints are frequently obtained. A clever extension of the use of reagent paper for the development of these negative prints is the electrographic or electro-spot procedure. This consists in making the solid test specimen (metal, alloy, mineral, etc.) the anode and a sheet of aluminum the cathode in an electric circuit. The electrodes are connected through a strip of filter moistened with the suitable reagent. On closing the circuit, metals from the alloy or compound dissolve at the anode. The metal ions travel toward the cathode but react on the way with the precipitation or color reagent in the pores of the paper. Typical colored spots are left on the paper and these may be used to identify particular substances that have dissolved at the anode. The use of an electric current for the production of ions is advantageous as the test can be carried out rapidly without visible damage to the specimen.

A general presentation of the theoretical foundations of spot test analysis, because it is a division of analytical chemistry, should necessarily take account of the ionic theory, the theory of dissociation, mass action effect, and so forth. The present discussion has, however, been limited to theoretical considerations concerning the chemical and physical processes having direct bearing on the significant "analytical effectiveness" of the chemical changes utilized in spot testing. *Analytical effectiveness may be defined as the characterization of chemical reactions by their sensitivity (identification limit and concentration limit), by their specificity or selectivity, and by their attainable limiting proportions.*

All measures for improving the analytical effectiveness of chemical reactions are of importance to spot test analysis in so far as they can be adapted to the special technic employed in this type of testing. Originally these tests were carried out by bringing together drops of the test and reagent solutions on filter paper or on non-porous surfaces. As a consequence of inclusion of all possible types of chemical reactions and of using physical means for improving the visibility of reaction products, it has become necessary at times to modify the earlier simple methods. In qualitative organic analysis particularly, the actual spot test is frequently made only as a sequel to preliminary preparative operations. However, all spot reactions have the common factor that one drop of the test solution is used, or that minute quantities of solid specimens are treated with one drop of reagent solution.

The first and most evident objective of spot test analysis is the microchemical detection or identification of small quantities of organic or inorganic materials by means of a simple procedure. This goes far toward meeting the objective of accomplishing analytical purposes with the most practical economy of material, time, and effort. Consequently, the range of application of spot testing is very diverse and extensive. Every analytical laboratory frequently can replace the laborious operations of the usual methods of qualitative analysis by simple spot reactions. This may be done not only without jeopardizing the certainty of the findings, but often the reliability of the results can in fact be greatly improved by this substitution. Speedy spot reactions can be applied with success to numerous special problems. Typical cases are: Tests of identity in the course of systematic qualitative analysis; preliminary tests preceding qualitative analyses; testing of insoluble residues; identification of minerals and ores; examination of technical materials, such as testing their purity. Spot test analysis also serves well in the biological sciences for the solution of medicochemical and physiological problems requiring chemical treatment. This also holds for the field of synthetic organic chemistry, where a check on building up and degradation reactions can be exercised by determining the presence, the disappearance, or the formation of certain characteristic groups. In conclusion, it may be noted that a series of didactic demonstration experiments designed to illustrate important chemical phenomena, processes, and properties can be presented in the form of spot reactions.

It has been found that the application of spot reactions is not confined to qualitative chemical analyses. Recently, color reactions that can be conducted in the form of spot reactions have been successfully employed for colorimetric determinations by comparing the color produced in one drop of the test solution with the intensity of the color developed in drops of different concentrations of a standard solution. This "spot colorimetry"

is a new and very simple form of quantitative microanalysis. The results are, of course, not as accurate as those attainable by the classical methods, but the accuracy is adequate for a preliminary orientation and for many technical tests, such as process control.

Spot test analysis would not have attained its present generally accepted importance in such a comparatively short time if its methods did not also have a theoretical basis. This foundation permits a scientific explanation of each operation and thus leads to purposeful improvements and extensions. The theoretical foundations have been indicated in the foregoing discussion, and an intelligent application of spot reactions demands an understanding of the chemistry involved, which sometimes is quite complicated. Consequently, a knowledge of the chemistry of surface reactions, that is, of capillary and colloidal chemical facts, is required, at least to some extent. Furthermore, complex-chemical reactions of all types play a very important part in spot testing, so that it is essential to understand the fundamental facts of the chemistry of complex-compounds. The manifold application of organic reagents requires a knowledge of organic chemistry, especially for the understanding of spot tests for organic compounds. Finally, physical chemistry cannot be neglected because the chemical changes involved in spot reactions conform to its laws. If the numerous possibilities of applying spot reactions to scientific and technical problems are kept in mind, it is evident that spot test analysis bears a close relation to many branches of chemistry and participates in their advancement, both with respect to its theoretical background and also its practical operation and application.

Spot test analysis thus impressively demonstrates the close union of all the separate branches of chemistry. This is of great didactic value. Although its external and most impressive effect is microanalytical in nature, spot test analysis, if properly understood, is not merely technic. Its entire essence is chemistry in all its branches and to this fact it owes its development as a line of research and a field of work.

Chapter II

THE TECHNIC OF SPOT TEST ANALYSIS

A. Laboratory

A special room should be assigned for spot test analysis in large research laboratories or for university courses in this branch of analysis. These quarters can well be a part of the microchemical division. A laboratory devoted to this type of work permits the convenient arrangement and installation of all apparatus, reagents, and other aids, as well as an undisturbed, clean working space. If no separate room is available, at least a certain part of the analytical laboratory should be reserved solely for working with spot test reactions. Good lighting of the work bench is particularly essential (daylight and daylight bulbs).

It is quite important to review the procedures before starting the examination of the samples, so that the necessary materials and apparatus will be at hand. Consequently, all equipment needed for frequently repeated operations should be installed permanently and all other appurtenances that are occasionally required should be set up nearby. Permanent installations include the centrifuge, Kipp gas generators, the analytical quartz lamp, devices for evaporation and ignition, and so forth. Glassware, porcelain vessels, reagents, and the like should be kept in separate places, arranged properly, and be easily accessible so that the work can be carried on most efficiently.

It is particularly important that the laboratory devoted to spot test analysis be kept scrupulously clean. There should be no dust and no acid and ammonia fog. Under such conditions, analytical balances and optical instruments can be set up in this room without hesitation. The ventilation and hoods must function efficiently. The water outlets and drains and the gas and electric installations must be adequate; a sufficient number of well-placed waste receptacles should be provided. The larger pieces of glassware and apparatus must be cleaned apart from the apparatus used in the actual spot tests. A separate bench should be assigned for filling the reagent bottles. A generous supply of well boiled cleaning cloths should always be available; they should not be used too long.

Although, as far as possible, only microchemical and semi-microchemical work should be done in the spot test laboratory, macrochemical operations cannot be avoided entirely. For instance, auxiliary reagents, as a rule, will be prepared in this laboratory, and the standard solutions will also be checked there. Considerable quantities of the specimen are sometimes necessary when testing for impurities or by-products. These larger quan-

tities are subjected to separation, concentration, ignition, and so forth, on a macroscale, to produce preparations or solutions, which can then be studied microchemically with the aid of spot reactions. Consequently, the design, arrangement, and equipment of a spot test laboratory must include facilities for qualitative and quantitative inorganic macroanalyses, for preparing materials that can be produced without too many complications, and for the separation of organic mixtures (extraction, distillation, etc.). A spot test laboratory designed for research and student purposes should also contain all the materials required for studies made by the classical methods of qualitative microanalysis. This is especially true because the choice of methods for a qualitative microanalysis should be determined, not by a dogged adherence to a particular technic, but by the desire to attain a certain microchemical goal as easily as possible.

The laboratory devoted primarily to spot test analysis must be provided with an abundant supply of chemicals. The ordinary reagents and the special materials necessary for the spot tests must be at hand. It is well to have a great variety of metal salts of the highest purity available. Oxides and carbonates, from which, if necessary, other salts can be prepared, are particularly desirable. The chemicals should be kept in small rather than large containers, to obviate contamination and decomposition. The same applies to the storage of spot- and fine reagents. Bottles of all sizes, vials, weighing bottles, and sample tubes must always be available for the storing of specimens and for the occasional preparation of auxiliary solutions and reagents. This glassware must be spotless and stored in closed cupboards. Gummed paper labels are a necessity.

It is well to assemble a collection of standard samples of chemicals, rocks, minerals, technical products, and typical organic compounds, which can be identified with certainty by spot test reactions. Permanent mounts of completed reactions should be included in this collection. All these items should be classified and indexed in a good card catalog. A collection of this kind often gives excellent service, particularly in difficult cases when a decision is reached by carrying out parallel tests. The preservation of standard preparations is also of great advantage for teaching purposes.

The spot test laboratory should also have a small working library. This should include the special literature of the field and reference books containing the necessary chemical and physical data. It will be found convenient to keep the books, catalogs, and so forth, on a book shelf over a writing desk near a blackboard.

The special laboratory whose plan is given on p. 266 is designed for about five chemists and one laboratory assistant. It can be used as a class room and for demonstrations before a larger audience.

A spot test laboratory should contain the following equipment and materials. They are classified under the headings:

1. *Reagents on the working bench and in storage vessels:*
   Wide-mouth bottles (250 g.) for solid reagents. See Appendix, p. 263.
   Auxiliary reagents (dropping bottles, storage flasks). See Appendix, p. 260.
   Spot reagent solutions (dropping bottles, storage flasks). See Appendix, p. 263.
   Organic solvents (alcohol, ether, acetone, carbon disulfide, amyl alcohol, pyridine).
   Indicator solutions (dropping bottles).
   Indicator papers (in wide-mouth bottles).
   Reagent papers (in wide-mouth bottles).
2. *Reagents in cupboards:*
   Organic reagents in wide-mouth bottles (arranged alphabetically).
   Stocks of salts of highest purity in wide-mouth bottles (arranged alphabetically).
3. *Glassware:*
   Beakers; Erlenmeyer flasks; suction flasks; round-flat bottom-fractionating flasks; Kjeldahl flasks; Liebig condensers; reflux condensers; distilling heads; watch glasses; Petri dishes; microscope slides; cover glasses; wash bottles; Woulff bottles; test tubes; centrifuge tubes, holders, and supports; crystallizing dishes; measuring cylinders; volumetric flasks; pipettes; burettes; funnels; separatory funnels; filtering crucibles and adapters; retorts; gas evolution apparatus (Kipp); extraction apparatus; desiccators; weighing bottles.
4. *Porcelain and quartz ware:*
   (a) porcelain: mortars; beakers; dishes; casseroles; suction filters; plates; crucibles with and without lids; Gooch crucibles.
   (b) quartz: dishes; crucibles; watch glasses.
5. *Metalware:*
   Water baths; sand baths; dishes; crucibles; tripods; ring stands; rings; clamps; burette holders; pipette holders.
6. *Woodenware:*
   Drainboards; filter stands; test tube racks.
7. *Special equipment:*
   See page 61.
8. *Miscellaneous:*
   Spoons; spatulas (porcelain, horn, metal); crucible tongs; test tube holders; tools (hammers, tongs, screw driver, penknife, scissors); forceps; cork borers; glass rod and tubing; thermometers; burners; rubber tubing; rubber stoppers; corks; brushes; stop watch.
9. *Balances:*
   Analytical; micro; trip scale.

10. *Electrical equipment:*
Drying oven; muffle furnace; hot plates; air heater; centrifuge; quartz lamp; refrigerator.

B. Working Methods and Special Aids

The term "spot reaction" is a generic name applied to microchemical and semi-microchemical tests for inorganic and organic compounds or for characteristic atoms and groups of atoms contained in them. These tests are accomplished by sensitive chemical changes which can be carried out directly or indirectly. In these tests an important part is played by manipulation with drops (micro or macrodrops) with no auxiliary optical magnification. Accordingly, spot reactions can be made:

1. By bringing together one drop each of the test solution and reagent on porous or non-porous supports (paper, glass, or porcelain).
2. By placing a drop of the test solution on a medium impregnated with appropriate reagents (filter paper, asbestos, gelatin).
3. By placing a drop of reagent solution on a small quantity of the solid specimen (fragments or pulverized; evaporation or ignition residues).
4. By subjecting a drop of reagent on a strip of reagent paper to the action of the gases liberated from a drop of the test solution or from a minute quantity of the solid specimen.
5. In an extended sense spot reactions may also include tests accomplished by adding a drop of reagent solution to a larger volume (0.5 to 2 ml.) of test solution and then extracting the reaction products with organic solvents.

The choice of the foregoing procedures will be determined by the nature of the sample, the reagents required, and the desire to attain the highest possible certainty and sensitivity of the test.

The actual "spotting" is the most essential manipulation in spot test analysis but it is not always the only operation involved. Frequently, preliminary measures are necessary to produce appropriate reaction conditions. Typical instances are: oxidation, reduction, adjustment to definite pH values, etc. Occasionally, in organic analysis it is even necessary to carry out syntheses on a small scale as a preliminary stage. Special expedients must sometimes be employed to make the reaction products as visible as possible (extraction, treatment with acids or bases). Accordingly, the technic of spot test analysis necessarily employs many of the familiar operations of macroanalysis but, of course, always on an appropriately diminished scale, and, so far as is feasible, without significant losses. Spot analysis, however, utilizes certain operations peculiar to itself and that cannot be applied at all in macroanalysis. These include

the employment of capillary surface actions on paper and other substrates (see p. 77) and the method of "spot colorimetry" (see p. 244).

The equipment and manipulations required in spot test analysis are all simple; the technic can be learned without difficulty. The essential requirements for a successful application of spot reactions include (1) a knowledge of the chemical basis of the tests so that the various steps of the procedures can be understood and executed intelligently; (2) strict observance of trustworthy experimental conditions; (3) scrupulous cleanliness of the laboratory and all equipment; (4) the use of the purest reagents. Whenever possible, tests should be carried out several times. In student exercises, where solutions of known content can be used, parallel tests should be made at various decreasing concentrations of the material being detected. The reaction picture is thus fixed in the learner's memory and, furthermore, he learns to appraise and compare the effectiveness of tests.

1. *Preparation and Storing of Solutions*

The preparation and storing of the solutions required for spot reactions vary with their particular purpose, their stability, and the frequency with which the solutions are to be used. Some solutions can be kept in readiness at all times, others must be prepared freshly when the occasion demands. Solutions of the test material, comparison solutions, and reagents which change on standing are examples of the latter class.

Stock solutions (see Appendix, p. 260) include the same auxiliary reagents that are used in macroanalysis: acids, bases, neutral salts; likewise, stable (water, alcohol, or acetone) solutions of the actual spot reagents; and, finally, organic solvents. It is very convenient to keep on hand water solutions of metal salts (nitrates or sulfates) and alkali salts of the various acids, containing 0.1 milligram per milliliter of metal or acid radical. These are used to prepare test solutions of the pure salt, or solutions of known dilution, for comparison when analyzing unknown materials. The determination of the sensitivity of certain procedures, the comparison of the sensitivities of various reactions, and also spot colorimetry require these standard solutions (see p. 263). They should therefore be accurately prepared from the purest chemicals. For this purpose, the calculated quantities of salts of analytical grade can be weighed out directly, and the solutions made up to the proper volume in calibrated flasks. For solutions made from hygroscopic or efflorescent compounds, the approximate quantity is weighed out, the solution made up to a known volume and the actual concentration then determined gravimetrically or titrimetrically. Neutral solutions of salts should be prepared, if possible. If acid must be added to prevent hydrolysis, as little as possible should be used, and the content of free acid noted on the label. Standard solutions of organic materials,

in organic solvents if necessary, can also be prepared for use in spot testing organic samples.

Considerable quantities of all stable solutions, such as auxiliary reagents, spot reagents, standard solutions should always be on hand. Since the actual spot reactions, as a rule, require only drops of the reagent, it is well to keep a small supply of all the necessary solutions on the work table in 50 ml. pipette- or dropping-bottles (Fig. 1). The storage bottles (2 liter for auxiliary reagents, 500 ml. for spot reagents, 1 liter calibrated flasks for the standard solutions) should be kept in a separate part of the laboratory. The small bottles can be filled from these with the aid of short-stem funnels.

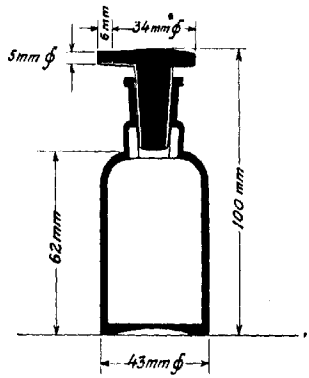


Fig. 1 (a). Dropping bottle
(½ actual size)

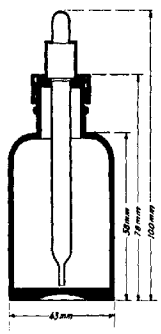


Fig. 1 (b). Pipette bottle
(½ actual size)

All bottles, as well as all glassware used in spot test analysis, should be made of the best resistant glass. Before being filled, the bottles must be cleaned thoroughly and steamed out. Alkaline solutions that may attack glass are best stored in bottles whose interior is coated with paraffin to about three-fourths of their height. Pure melted paraffin is poured into the bottle, which is then steadily swirled while it is being lowered up to its neck in boiling water. The coated bottle is allowed to cool in the air.

All dropping- (or pipette-) bottles should be labeled plainly as to the nature and concentration of their contents. It is convenient to keep the auxiliary reagents, solvents, and spot reagents in racks or blocks on the work table; the auxiliary reagents are grouped according to acids, bases, and neutral salts, and the actual spot reagents arranged alphabetically.

Solutions should be taken from the small bottles only by means of their pipettes or through the channel in the neck and stopper; never by pouring.

Since the drops delivered by the pipettes or dropping bottles are often too large, it is well to transfer several drops from these bottles to a "solution dish" (Fig. 2) and then take the reagent from this by means of a glass rod, pipette, or the like. The solution dishes are glass beakers with 1 millimeter walls and a capacity of 1 to 2 milliliters. A considerable stock of these should always be on hand.

As soon as solution dishes have been used, they should be put into basins filled with water. The basins should have handles, and separate basins should be provided for acid, basic, and neutral solutions. To clean the dishes, the water is poured off, and the basins containing the dishes are placed under the tap for a while. The dishes are finally rinsed several times with distilled water. The dishes can be cleaned quickly in this way; they are then placed on a double layer of filter paper and allowed to drain and dry in the air.

If a solution cannot be used at its original strength, but must be diluted, this can be done, depending on the desired accuracy, either in a graduated

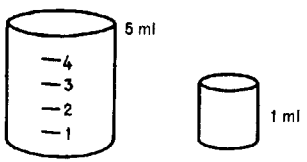


Fig. 2. Solution dishes
(actual size)

cylinder (5 to 25 ml.) or with the aid of a standardized pipette and volumetric flask. When an approximate dilution will suffice, or if several water solutions of salts are to be mixed to carry out exploratory trials, one drop of the solution to be diluted, or of the salt solution to be mixed, can be placed in the depression of a spot plate. The necessary number of drops of water or of the other aqueous solutions are added and the solutions then made uniform by blowing into them through a dry pipette, or by stirring with a fine glass drop. The drops for the spot reactions and like purposes can then be withdrawn directly. This is a rapid procedure and suffices for many purposes.

Solutions of solid samples that are to be subjected to spot reactions can be prepared in microcrucibles or test tubes. The smallest feasible volume of the suitable solvent (chosen or found by trial) should be used. The circumstances prevailing in each case will determine whether such solutions can be tested without further manipulation or whether the undissolved portions of the sample or the excess solvent must be removed before proceeding. This point requires special consideration. As a rule, it is unwise

to test solutions that are too strongly acid or too alkaline. Consequently, and especially when dealing with acid solutions, it is often necessary to remove the excess acid.

Test solutions which are not to be used immediately should never be allowed to stand uncovered on the working table. This is particularly important when small quantities are involved. The crucible can either be covered with a small watch glass or placed in a small desiccator, whose lower compartment contains a dish filled with water. Undesirable evaporation can thus be avoided. On the other hand, if cautious evaporation at ordinary temperatures is desired the crucible is allowed to stand overnight in a desiccator charged with solid calcium chloride or concentrated sulfuric acid, or a small vacuum desiccator may be used (see also p. 46).

2. *Grinding and Mixing Solids*

Solid specimens must often be subjected to definite chemical changes or special treatment in the course of analytical examination. These processes include solution in water, acids, or alkalies; fusion with disintegrating agents (sodium carbonate, peroxide, pyrosulfate); volatilization of certain components (fuming with hydrofluoric acid, sulfuric acid, etc.); extraction with organic solvents. The maximum reactive surface of the solid should be provided in such cases so that rapid and complete reactions may occur. This requirement is met in the case of slightly soluble compounds only when they are freshly precipitated and gently dried if need be. Ignited materials, especially natural or technical products, as a rule, must be pulverized beforehand, since a direct testing on compact surfaces for purposes of identification is only feasible in exceptional cases.

Samples are best pulverized in agate mortars. A set of these with different capacities (0.5 to 6 ml.) should be provided. Mortars and pestles of good porcelain or glass are less resistant but are good enough for many purposes. Very hard specimens should be broken up in a steel diamond mortar before being pulverized in agate. Fig. 3 shows a micromortar of correct dimensions.

Mere grinding of hard materials in mortars usually produces a mixture of aggregates of different sizes which must be homogenized before further treatment. This is accomplished by bolting the powder through a tightly stretched fine silk cloth. When the bolting cloth is tapped, the finest particles of the powder fall through the meshes and the coarser fragments remain behind. The latter are reground and the sifting and grinding continued until the *total* sample has passed through the cloth. Only when a perfectly homogeneous material is being examined may the coarser residue be rejected and the first portion that has passed through the sieve be taken for the chemical examination.

The arrangement shown in Fig. 4 is suitable for bolting small samples. It consists of an open glass tube with a flared rim. The tube is easily obtained by cutting off the bottom of a wide hard glass test tube. A circle of very fine silk is stretched over the end and fastened with a string. The powdered sample is placed in the center of the cloth, and the open end of the tube is pressed against a square of white or black glazed paper. The cloth is then tapped at the side of the powder with a glass rod. After the fine powder has passed through the silk, the paper is folded into a V and the powder brushed into a crucible or other reaction vessel.

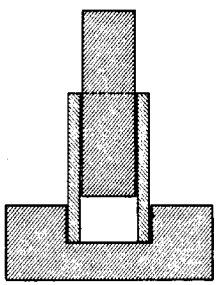


Fig. 3. Micromortar
(actual size)

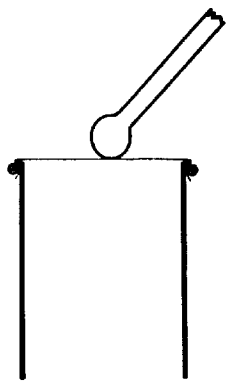


Fig. 4. Microsieve
(actual size)

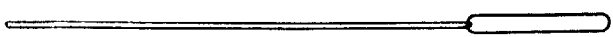


Fig. 5. Microspatula (nickel)
(actual size)

Pulverized samples are mixed with powdered reagents only if the materials are well dried. The mixing can be done in crucibles, on watch glasses, and frequently on squares of glazed paper. Dry powders, that are not hygroscopic, can be weighed satisfactorily on tared sheets of glazed paper and can be brushed off the paper quantitatively. Micro-spatulas, platinum wires, or thin glass rods are suitable for mixing powders; wooden splints (toothpicks) often can be employed for this purpose. Paper and toothpicks offer the advantage that they can be thrown away after they have been used once. Small horn spoons are used for transferring powders; nickel micro-spatulas (Fig. 5) are employed for very small quantities. Dis-

secting needles, a good magnifying glass, forceps, streak plates of unglazed porcelain (for testing the hardness and streak of a powder), and small weighing bottles for storing specimens, are among the other aids required for handling and working with solid samples.

3. *The Taking and Application of Drops*

The taking of drops from a test solution or reagent can be done in various ways depending on the particular conditions surrounding the experiment. The simplest method is to plunge a glass rod into the solution and then let a drop fall off. The size of the drop depends on the thickness of the rod (these are usually 20 centimeters long). A glass rod 3 millimeters thick delivers drops whose volume is about 0.05 ml., while smaller drops are delivered by rods approximately 1 millimeter thick. The use of glass rods is, however, permissible only for orientation trials, because it is impossible to regulate the size of the drops. If the rod is not wetted sufficiently, there is danger that the drops flow off too slowly, and the operator is tempted to touch the filter paper or other substrate with the rod. This results in the liquid being sucked off the rod with resultant loss of control of the size of the drops. If a precipitate is being formed, parts of it may stick to the rod, and in some cases this impairs the typical reaction picture.

An exact controlled delivery of drops can be secured by using pipettes. Simple glass pipettes about 20 centimeters long can be made easily by drawing out tubes of resistant glass (4 mm. inside diameter). The width of the capillary constriction determines whether macrodrops (0.05 ml.) or microdrops (up to 0.001 ml.) are delivered by the pipettes. Pipettes with longer (up to 5 cm.) drawn-out capillary ends (inside diameter 1 to 0.5 mm.) are suitable for smaller volumes of liquid and the delivery of smaller drops. The transfer of smaller volumes of liquid from microcrucibles, centrifuge tubes, and so forth can be done with the dropper pipettes shown in Fig 6.

When drops are delivered from pipettes, care must always be taken that their outer surfaces are dry so that excess liquid is not carried along. This can be avoided by wiping the filled pipette with soft filter paper, which is then discarded.

If liquids are drawn into a pipette by mouth, the pipette should be held so that the index finger is free and is kept so that it can easily be placed over the end of the pipette. The thumb presses the tube of the pipette against the three other fingers. The capillary end of the pipette is placed in the liquid, the operator inhales slowly taking care that no air bubbles get into the capillary. The pipette is then closed quickly with the index finger. The finger must not be clamped down tightly but should exert only a slight pressure. The drop-wise delivery of solutions from the pipette is accomplished by a very slight, partial raising of the finger. A

little practice will give the correct and confident feeling for delivering single drops. A pipette must never be discharged or closed with wet fingers because of the danger of slippage with consequent loss of control of the delivery of drops. The taking and delivery of liquids and drops by means of a dropping pipette provided with a rubber cap must also be practiced to secure complete mastery in the handling of drops. The measurement of the volume of drops is discussed on p. 64.

When drops of liquid are placed on paper or spot plates by means of pipettes, the latter should be held at a very obtuse angle to the horizontal

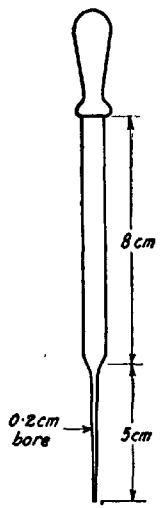


Fig. 6. Dropper pipette
(½ actual size)

surface. The capillary point should not be more than 1 centimeter above the place where the drop is to be delivered. If drops of the test solution and the reagent are to be brought onto filter paper one after the other, care must be taken that the second drop falls as nearly as possible in the center of the first drop. However, sometimes there are particular reasons, such as to secure better visibility of the reaction, for spotting from the side.

It is often an advantage when carrying out spot reactions on reagent paper not to drop the test solution onto the paper, but to flow it on from a capillary pipette by lightly pressing the tip onto the paper. The solution

then spreads concentrically from the point of contact; dissolved materials or reaction products are thus retained in the immediate vicinity of this point.

Drops of a reagent may be taken directly from the pipette or dropping bottles in which they are stored, but it is always preferable to transfer a small volume of the reagent to a solution dish (see p. 29) and then to take the desired quantity from this by means of a pipette. This procedure offers the advantage that any necessary dilution of the reagent can be made in the dish.

An adequate number of glass rods and pipettes, in sufficient variety of sizes, should always be kept in readiness. It is best to store pipettes with the constricted ends down. Porcelain beakers about 10 cm. high, contain-

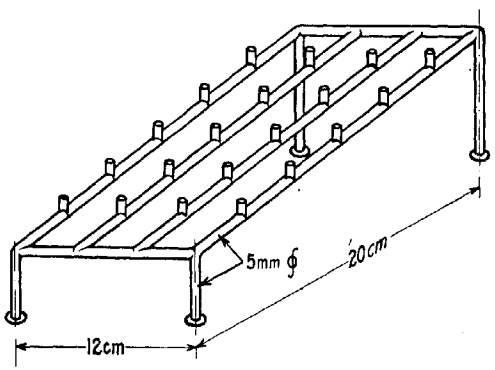


Fig. 7. Support for pipettes, rods, etc.
(¼ actual size)

ing a layer of cotton to prevent chipping of the glass, are excellent holders for pipettes and glass rods. They and their contents can be protected against dust by keeping them under cellophane shields.

Pipettes should never be laid on the work table lest they become dirty, or their contents trickle out. If the same pipette is used several times for parallel tests, it is best to lay it on a holder (Fig. 7), easily made from glass rod. As soon as pipettes and glass rods have been used, they should be plunged into beakers filled with water. This prevents interchanges. It also facilitates cleaning, because solutions easily evaporate on the glass, particularly in the capillaries, and may leave salt crusts.

Very small and uniform drops are obtained by means of platinum wire loops. The size of the loop can be varied, and the corresponding volume of

liquid delivered can be determined fairly accurately by weighing the drops. The loops, of different sizes, are made by bending platinum wire of proper thickness and soldering the junction. A handle is provided by inserting the wire in the usual manner into a length of softened glass rod or tubing. These wires are kept in Pyrex test tubes provided with corks or rubber stoppers; the labels should show the size of the drop delivered. Smooth new platinum wire allows liquids to drip off too readily, and hence it is necessary to roughen the wire by dipping it into platinic chloride solution and

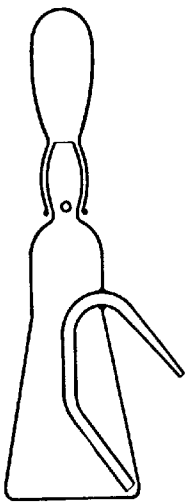


Fig. 8

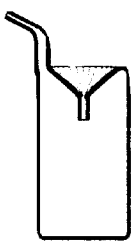


Fig. 9

Fig. 8. Storage bottle for solutions, which permits controlled delivery of drops (½ actual size)

Fig. 9. Device for delivering drops of water (capacity = 30 ml.)

then heating it to redness. This treatment should be repeated several times.

A storage bottle for water and solutions, which do not deteriorate on standing, and permitting easy delivery of drops, is shown in Fig. 8. It consists of a Pyrex flask into which a tube with a capillary end has been fused. The constricted neck, which is provided with a hole to admit air, is closed with a small rubber bulb.

A simple and practical apparatus for delivering drops of water is shown in Fig. 9. It has a capacity of about 30 ml. It is filled through the funnel-

shaped arm, while suction is being simultaneously applied through the side arm by means of a water pump.

Fig. 10 shows a small apparatus for delivering uniform drops of mercury. It consists of a storage vessel and is closed by a glass cock whose plug has a depression (A) in place of the usual bore. The size of the drop is determined by the size of (A) and, consequently, different sized drops can be delivered by varying the depression. One advantage of this "mercury dropper," which can also be used to deliver drops of other liquids, is the tight closing of the storage vessel and the unvarying size of the drops discharged. The vessel is filled by taking out the cock and inserting a short-stem funnel in the delivery tube.

Burettes can also be used to deliver drops. In qualitative spot test analysis, it is well to use microburettes with a capacity of 0.5 to 1 ml. when sensitivity determinations are being made, or when the size of drops is being

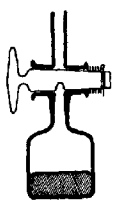


Fig. 10. Apparatus for delivering uniform drops of mercury
(½ actual size)

determined as described on p. 65. Microburettes are indispensable in spot colorimetry (see p. 245).

4. *Spot Reactions on Filter and Reagent Papers*

Spot reactions are performed either by bringing together a drop of the test solution and of the reagent on filter paper, or by placing one drop of the test solution on filter paper impregnated with an appropriate reagent. Not all papers are suitable for these operations and the quality of the paper must therefore be considered in every case. The purity of the paper is an important factor, but the almost complete absence of mineral constituents, which may be considered primarily as contaminants, is frequently less significant than the correct structure of the paper, that is, its thickness, texture, permeability, and shape of the fibers, which determine the absorbability.

The most frequent contaminant is ferric iron. This is detected easily by placing a drop of acidified, saturated solution of potassium thiocyanate on the paper; on drying, a red spot or ring of iron thiocyanate will be seen.

Practically all varieties of paper contain minute quantities of silica; also traces of barium, calcium, magnesium, and, occasionally, phosphate. Alkali metals, particularly potassium, are found only in qualitative papers. They are absent in good qualitative papers which have been washed with acids and water.[1]

Practically all filter papers contain small quantities of starch. It should also be noted that the cellulose of filter paper is by no means an inert material. It can be oxidized and can, therefore, act as a reducing agent. The relatively small moisture content of properly stored filter paper does not interfere with spot reactions, but it must be remembered that strong caustic alkalies and concentrated acids rapidly swell the fibers or make them brittle. Consequently, filter paper, as a rule, is suitable only for working with solutions that are not extremely acid or basic. The purity and resistance towards chemicals of most of the good papers on the market are adequate for the majority of spot reactions. This is not true, however, with respect to the requirement of suitable imbibition.[2]

It is particularly important that the paper soak up liquids rapidly, but on the other hand, the drop placed on the paper, the so-called "spot" or "fleck", should not spread too much. (See page 65 regarding the determination of the absorptive powers of paper by measuring the iodine picture.) The typical reaction picture of a spot test on filter paper always appears within the bounds of the spot, and consequently too much spreading interferes with the detection of small quantities of colored reaction products. Very thin filter paper, on which capillary spreading results in spots that are too large, is therefore inherently unsuited for spot reactions.

The capillarity of the paper is responsible for the fact that the sensitivity of spot reactions is often greatly dependent on the type of paper employed. Consequently, it is important to use types or varieties of paper that are preeminently suited to the purpose, when minute quantities of material are being sought (especially in the vicinity of the identification limit), and when utilizing capillary separations in paper (see p. 77). Many spot reactions have been worked out with the aid of the following Schleicher and Schüll papers. Whatman papers give approximately the same results.

[1] The mineral constituents of filter paper come from the inorganic materials contained in the cellulose fibers and from the salts in the well water used in the manufacture of the paper. The ash content of the better grades of qualitative paper, that have not been washed with acids, averages 10 γ per square centimeter. "Ash free" papers, which have been extracted with hydrochloric and hydrofluoric acids and then washed with distilled water, have an ash content of 0.8 γ per square centimeter; about half of this is silica.

[2] The differences in imbibition shown by various kinds of filter paper are a result of the nature of the raw materials (linen and cotton fibers), the method of beating, and the drying of the finished moist paper. The shorter the fibers and the lower the drying temperatures, the softer and more absorbent the paper.

The following papers may be regarded as equivalent:

Schleicher and Schüll		Whatman	
No. 601	"Spot Paper"	No. 120	Drop reaction paper, double thickness
598		3MM	1st quality
589		42 or 542	

Filter papers intended for spot reactions should be stored in vessels with tight lids, in boxes, or in Petri dishes. The type of paper should be stated on the label of the container. The paper should be cut into strips (2 × 2 cm. or 2 × 6 cm.); these make for economy and convenience. Beginners are urged to carry out spot reactions with dilute solutions under constant conditions on various types of filter paper to acquire a background of experiences.

Filter paper should not be held in the hand, nor laid on the table or on a paper, when drops are placed on it from pipettes and so forth. Losses due to run-off and imbibition by the support should be avoided. The proper method is to lay the strip across an open porcelain crucible and to place the drops in the middle of the horizontal paper. An unhindered capillary spreading follows and circular spots result. A number of comparison spots can be produced if a longer strip and a larger crucible are used.

Spots are dried by allowing the strips to remain on the crucible, or the entire set-up is placed in a drying oven or subjected to a current of warm air (see p. 53). So far as possible, manipulations with filter papers should be accomplished without contact with the fingers. If it is necessary to dry the strip in a current of warm air, or to plunge it into an acid bath, or to subject it to the action of a gas, one corner of the strip should be held with a pair of forceps. Obviously, care must be taken not to bring the forceps into contact with the moist spot, nor with acids, etc.

Spot reactions on paper do not always involve the union of a drop of the test solution and one of the reagent. Sometimes filter paper is impregnated with the proper reagent and the dry reagent paper is spotted with a drop of the test solution. This procedure, which assumes, of course, the availability of stable reagents, has the advantage that there is no mutual dilution of the reagent and test solution. A better localization and visibility of the reaction products at the place where the spot has formed is achieved, as compared with the result of bringing two drops together. A still better effect is obtained by impregnating filter paper with reagents that are so slightly soluble in water that no bleeding occurs when a drop of the test solution strikes the paper. Organic reagents that are only slightly soluble in water, but that dissolve readily in alcohol or other organic solvents, have this advantageous characteristic. Slightly soluble compounds, that can

be precipitated on paper and in its capillaries by certain chemical reactions, can also be used in this way.

Spotting on reagent paper impregnated with an insoluble compound involves a reaction of dissolved materials with an insoluble reagent. This procedure cannot be used in macroanalysis because compact materials, in general, react too slowly. If, however, these same solids are finely divided by precipitation in the capillaries of paper and thus endowed with an extensive reactive surface they will undergo chemical changes almost as rapidly as soluble reagents (see p. 63). The localization of characteristic reaction products, with consequent better visibility and increase in the sensitivity of the test, is not the sole advantage of using reagent papers impregnated with insoluble compounds. In many cases, a highly desirable homogenizing and stabilization can be accomplished by impregnating filter paper with insoluble compounds which then behave like soluble materials. For instance, it is not possible to prepare a good stable alkali sulfide paper; it oxidizes to sulfate too rapidly and the highly soluble alkali sulfide is washed away when the paper is spotted with an aqueous solution. On the other hand, it is easy to impregnate filter papers with slightly soluble sulfides (ZnS, CdS, Sb_2S_3, etc.). Such papers are stable; each has its maximum sulfide ion concentration (controlled by its solubility product) and hence precipitates only those metallic sulfides whose solubility products are sufficiently low. Antimony sulfide paper precipitates only silver, copper, or mercury in the presence of lead, cadmium, tin, iron, nickel, cobalt, and zinc. Another striking example is the detection of iron by spotting on paper impregnated with the difficultly soluble white zinc ferrocyanide. In this form the test is far more sensitive than when it is made by uniting drops of a ferric solution and an alkali ferrocyanide, or by spotting on potassium ferrocyanide paper. The latter also is less stable than zinc ferrocyanide paper. Consequently, if possible, *it is always better to impregnate filter paper with "insoluble" reagents than with soluble ones.*

It is easy to impregnate filter paper with reagents that are soluble in water or in organic solvents. The proper solutions are prepared in beakers or dishes and the strips of filter paper are bathed in them. Care must be taken that the strips do not cling to the sides of the container, that they do not touch each other or stick together, because this may prevent a uniform impregnation. The immersion should last for 20 to 30 minutes, the solution should be stirred quite frequently, or the vessel swirled, to produce uniformity. The strips are taken from the bath, allowed to drain, pinned to a cord (stretched horizontally) and allowed to dry in the air.

Instead of soaking the strips in the solution, reagents can be sprayed onto filter paper. The atomizing tip shown in Fig. 11 is excellent for this purpose. The impregnating solution is placed in a wide test tube which is

then closed with the atomizing head. The paper is held horizontally and the spray expelled by blowing into the apparatus. The paper is sprayed first on one side and then on the other.

Filter paper is impregnated by soaking it in the appropriate solutions or by spraying when it is desired to prepare a stock of reagent papers. The following procedure is recommended for single experiments or when, for special reasons, the spot produced by a drop of a solution must be dried before adding a drop of the other reactant. V-shaped strips of filter paper are spotted on each side, taking care that the spots stay in the center of

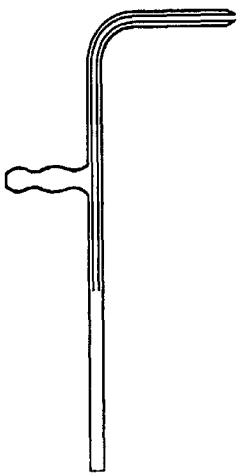


Fig. 11. Atomizer head for spraying reagents
(½ actual size)

the strips as nearly as possible. The strips then remain so stiff that they can be stood on the table and allowed to dry. The impregnated strips are cut at the crease before they are used.

Homogeneous impregnation can only be accomplished through gradual uniform drying on all sides. If paper is soaked with a salt solution and then dried by exposing it to a stream of heated air from the drying apparatus, the rapid evaporation and the subsequent capillary diffusion always leads to an accumulation on the side of the paper turned toward the blast. This effect can be detected immediately in the case of colored reagents, because the color is far less intense on the side of the paper turned away from the blast. The localization of a reagent on one side of the paper is an advantage, par-

ticularly for water-soluble reagents, because it is desirable to have the largest possible quantity of reagent available at the place where the spot is made, so that there will be a rapid and complete reaction with the materials in the test drop.

If strips of filter paper are dried in a blast of hot air, they should either be held on both ends with forceps, or laid on a ribbed porcelain plate at such distance from the hot air apparatus that the current of warm air presses the paper against the porcelain plate. The completion of the drying is determined easily because the moist paper, which adhered to the plate, rises and flutters in the current of air.

There are no general procedures for impregnating filter paper with compounds produced by precipitation on and in the paper. As a rule, the strips are soaked with the solution of one of the reactants, dried, and then immersed in the solution of the appropriate precipitant. The excess reagents are removed then by washing, and the paper dried. If this method is used the order in which the solutions are applied, as well as their concentrations, makes a difference. The best conditions must always be determined by trial. It is preferable to use a reagent if possible in the gaseous form (hydrogen sulfide for precipitating sulfides or ammonia for precipitating oxides) rather than in solution; there is then no danger of washing away the precipitate. For the same reason it is often advantageous first to form an adherent compound which cannot be washed off the paper and then carry out the reaction producing the desired reagent. For instance, a good lead sulfide paper is produced—not by soaking the paper in a lead salt solution followed by treatment with hydrogen sulfide water or gas—but rather by forming zinc sulfide on the paper and converting this into lead sulfide by bathing in a solution of a lead salt.

It must be remembered that few reagent papers are as stable as the familiar indicator papers, which owe their permanence to an irreversible adsorption of dyes on the paper. In general, the impregnation with amorphous compounds is more permanent than with crystalline materials. The latter recrystallize, their surfaces are diminished, they become detached from the capillaries of the paper and the original homogeneity and high reaction rate are lost. The decreased activity of old reagent papers is due therefore, in general, not to chemical but to physical (surface) changes. These occur to quite different degrees with different compounds. In most cases, careful storage in closed containers and protection from light will insure adequate stability for several weeks.

5. *Spot Reactions in Porcelain and Glass Vessels*

In the performance of spot reactions, and particularly in the course of preliminary operations, it is often necessary to use glass, porcelain, or metal apparatus. These are required to carry out, on a small scale, certain op-

erations, such as treatment with concentrated acids, caustic alkalis, ignitions, fusions, etc.

The use of non-porous materials is especially imperative when one (or more) of the reactants is so strongly alkaline or acid that a rapid swelling or weakening of the fibers of filter paper is likely. In such cases, it is convenient to use spot plates (Fig. 12). These may be either white or black and made of glazed porcelain or resistant glass. They have 6 to 12 identical depressions, each with a capacity of 0.1 to 1 ml. An adequate supply of spot plates with different sized depressions should be kept on hand. The white porcelain background makes it possible to detect very small differences in color intensity or slight changes in color. This is particularly true if parallel blank tests with drops of water are made in adjacent depressions. Comparison tests with drops of solutions of known content can also be carried out in adjoining depressions (see Spot Colorimetry, p. 249). Black

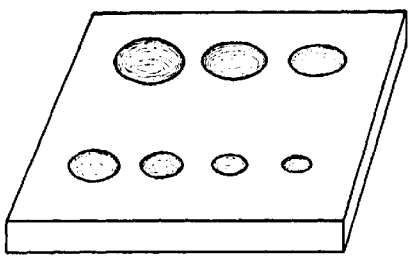


Fig. 12. Spot plate with depressions of different capacities
(½ actual size)

spot plates are an advantage if light colored (or colorless) precipitates or turbidities are formed. Glass spot plates are excellent. They can be placed over glossy paper of various colors; thus the observation of minute quantities of precipitate or soluble colored reaction products is often made easier.

The drops of test solution and reagent brought together on a spot plate must always be well mixed. This is done with a glass stirrer (Fig. 13) or, still better, by blowing onto the solution through a fine pipette. During the blowing, the end of the pipette is held about 0.5 cm. away from the surface of the liquid and moved over it.

Even a slight coating of grease in the depression of the spot plate will prevent drops from coalescing. In delicate tests with microdrops it is therefore imperative to clean the depressions by wiping them with cotton moistened with a mixture of alcohol and ether.

Precipitates that are formed by the addition of reagents dissolved in alcohol or ether will creep up the walls of the depression as the organic solvent evaporates. The same is true for the dissolved reagent and, in case it is colored, blank tests are necessary without exception.

Porcelain is attacked on long contact with alkaline solutions and the smooth surface becomes rough. Careless cleansing may also damage porcelain. These evils may be avoided if it is made a matter of routine not to allow the spot plates to stand after use nor to permit solutions to dry up in the depressions. The plates should be placed, as soon as possible, in glass dishes filled with water. This prevents troublesome adherence of precipi-

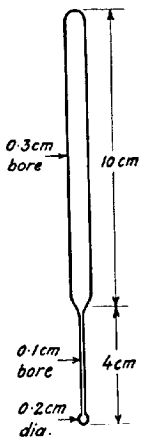


Fig. 13. Stirrer (glass)
(½ actual size)

tates and incrustations and, as a rule, the plates can be cleaned completely by merely swabbing them with a cloth. In case the porcelain still retains the precipitate, a suitable acidic or basic solvent is used. This should be as dilute as possible. Spot plates should not be cleaned with the ordinary chromic acid solution; it attacks them badly.

Some spot reactions can be carried out on paper just as well as on spot plates and with equal sensitivity. Often, however, a typical reaction product can be detected more easily, and in smaller amounts, on one, rather than the other of these backgrounds. Spot tests on paper are more sensitive and consequently are preferable when adsorption or other capillary action of the paper, that lead to an accumulation and localization of colored reac-

tion products, are involved. On the other hand, weakly colored compounds, particularly yellow ones, or slight color differences produced by colored reagents, generally can be distinguished more readily on spot plates. The beginner is urged to try tests on a spot plate even though the directions recommend the use of paper, and *vice versa*. He will thus obtain his own opinion of the characteristic reaction picture and the range of application of the two most important methods of conducting spot reactions.

If a comparison with blank tests is not necessary, spot reactions can be made on small watch glasses, in microcrucibles (Fig. 14), in the bowl of a small porcelain spoon, or on a grooved piece of magnesia. Crucible covers or watch glasses made of quartz or quartz glass are sometimes suitable. A drop of solution can be easily evaporated or even ignited on them as a preliminary to the actual spot reaction.

Microcrucibles are excellent if solids must be brought into solution by digestion with acids or alkalies preparatory to the spot reaction, or when a small volume of liquid is to be concentrated or evaporated. The test tubes

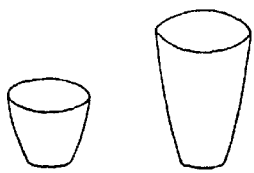


Fig. 14. Microcrucibles
(actual size)

shown in Fig. 15a serve the same purpose. By properly inclining them it is possible to dissolve small quantities of alloys and similar materials, make the evaporation, take up the residue in water, and deliver drops of the resultant solution through the side arm onto filter paper or a spot plate.

Spot reactions that produce tiny precipitates or faint turbidities, or those requiring subsequent extraction with organic solvents to concentrate the reaction product are conveniently carried out in micro-test tubes, either open or provided with glass stoppers (Fig. 15b). Solutions can be heated or boiled in these without serious loss due to evaporation or spattering. Such micro-test tubes should be made of resistant glass, kept in suitable racks, and cleaned with fine brushes. Turbidities or colors can easily be seen in these test tubes when viewed against a white or black background. Rectangles (6 × 12 cm.) of black or white glazed paper or lusterless cloth pasted on thick cardboard are excellent for this purpose. Dull aluminum foil sometimes can be used to advantage.

If small quantities of precipitates are to be made visible, it is well to make

the spot reaction in the glass tubes shown in Fig. 16, and to centrifuge after allowing the reaction mixture to stand for a while. The precipitate collects in the capillary end of the tube, and can be detected with a magnifying glass if viewed against a suitable background.

Fig. 15 (a)

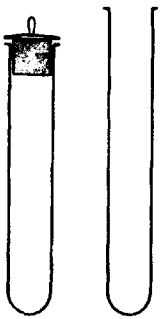


Fig. 15 (b)

Fig. 15 (a). Special test tube for solution of sample and delivery of drops of the resulting solution
Fig. 15 (b). Micro-test tubes
(actual size)

Fig. 16. Centrifuge tube
(½ actual size)

Fine platinum wires are used to stir solutions in crucibles, micro-test tubes, or centrifuge tubes. The glass stirrers, whose proper dimensions are shown in Fig. 13, are excellent.

The methods recommended for cleaning spot plates should also be used for crucibles, micro-test tubes, and the like. They should be put into water immediately after use. Care must be taken that all glass apparatus is thoroughly dry and free from dust and grease.

6. *Evaporation—Ignition—Fusion*

Concentration of solutions, that is, diminution in volume by removal of most of the water, evaporation of solutions to dryness, or removal of volatile acids by warming, are carried out chiefly in porcelain or platinum microcrucibles. The apparatus shown in Fig. 17 is very convenient for all these operations. It consists of an aluminum block provided with wells for a thermometer (T) and for two or three microcrucibles. A small glass bell with a stop cock is fitted tightly to the block. With this apparatus it is also

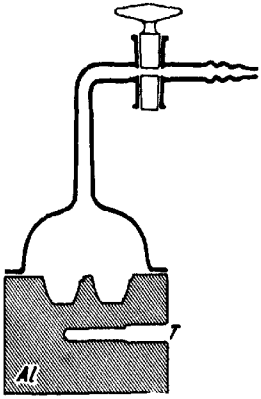


Fig. 17

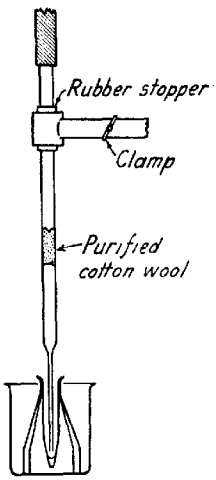


Fig. 18

Fig. 17. Aluminum block for concentrating solutions, etc. (½ actual size)

Fig. 18. Set-up for concentrating a solution in a centrifuge tube (½ actual size)

possible to remove water and volatile compounds under reduced pressure and at temperatures below the normal boiling points. If the bell is placed on a well-fitting ground glass plate, the assembly can be used as a microdesiccator.

Small volumes of liquid can be concentrated or taken to dryness in centrifuge tubes at water bath temperature by blowing in dry filtered air. This is sent through a glass capillary and impinges on the surface of the liquid to be avaporated. Details of the apparatus for this operation are shown in Fig. 18.

If water bath temperatures are not sufficient to remove volatile com-

pounds, air or sand baths can be employed to furnish the higher temperature required. A simple air bath is shown in Fig. 19. It consists of a nickel crucible with a copper wire triangle suspended in it through lateral slits. The triangle supports a microcrucible or microbeaker. The nickel crucible can be heated directly by a burner or placed on a hot plate. The tempera-

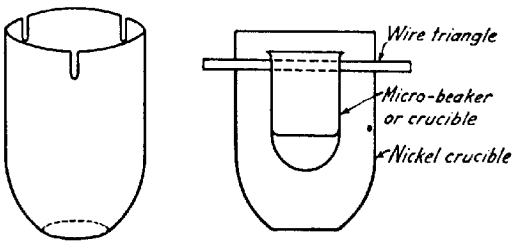


Fig. 19. Air bath constructed from nickel crucible
(½ actual size)

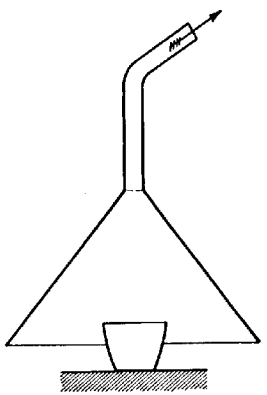


Fig. 20. Set-up for leading fumes away
(½ actual size)

ture can be determined by suspending a thermometer in the crucible. If filled with fine sand, the crucible can be used as a sand bath. In this case the vessel containing the liquid to be evaporated is placed on or in the sand. The apparatus should be set up in a hood if clouds of acid vapors or ammonium salts are expected. An alternative arrangement is to place a glass funnel (see Fig. 20) over the crucible and draw the fumes away with a good filter pump. The heating bath can then be set up on the laboratory bench.

A silica watch glass can be used when evaporating small volumes of liquid and igniting the dry residue. This has the advantage that the evaporation or ignition residue can be inspected directly, particularly when the watch glass is held against black glazed paper.

Products can be ignited or ashed in porcelain or platinum crucibles, on silica watch glasses, or on a piece of grooved magnesia (Fig. 21). The direct flame of a microburner is used. Ashing and ignition are advantageously carried out in electrically heated furnaces provided with temperature indicators. The action of the gases from the burner and losses through spattering and decrepitation are thus avoided.

The examination of silicates and other materials, that are not soluble in acids, often necessitates fusion with a disintegrating flux. Oxidizing fusions are sometimes required. Small quantities of the sample can be mixed with sodium-potassium carbonate, sodium bisulfate, or alkali peroxide and then

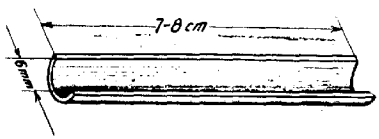


Fig. 21. Grooved magnesia for ignitions (actual size)

Fig. 22. Platinum spoon, with handle fused into a glass tube (actual size)

heated in the loop of a platinum wire. A small platinum spoon, capacity about 0.5 to 1 ml., that has its handle fused into a glass tube (see Fig. 22), is highly recommended for these fusions. Silicates can also be decomposed in a platinum spoon by fuming with hydrofluoric and sulfuric acids; volatile silicon tetrafluoride is formed. The residue can be readily examined or subjected to further treatment. A magnesia groove or rod sometimes suffices for the decomposition of certain samples.

7. *Action and Liberation of Gases (Vapors)*

Two kinds of manipulations with gases or vapors are used in spot test analysis. Gases (vapors) are employed as auxiliary reagents for precipitation, alkalization, or oxidation of solutions. On the other hand, the liberation of small quantities of gases (vapors) as characteristic products which can be identified by subsequent reactions is the basis of certain tests. The

apparatus required for handling gases (vapors) is determined by the purpose at hand.

Spot reactions on paper involving the action of gases or vapors (H_2S, NH_3, halogens, steam) can be conducted by leading the gas directly from the generator, or by placing a strip of filter paper over the neck of an open flask filled with hydrogen sulfide water, ammonium hydroxide, etc. The steamer (Fig. 23) can be used as a gas generator, if the flask is filled with hydrogen sulfide water, bromine water, or ammonia water, warmed, and the material to be gassed placed on the side arm.

The separation of certain groups of metals by treating the acid or ammoniacal solution with gaseous hydrogen sulfide is a common step in chemical analyses. In spot test analysis, this precipitation can be accomplished by

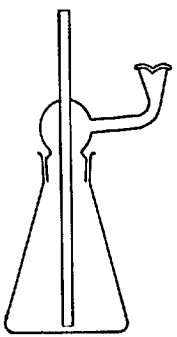


Fig. 23. Apparatus for treating paper with gases or vapors (actual size)

saturating a small volume of the solution with hydrogen sulfide in a microcentrifuge tube. The hydrogen sulfide is admitted through a fine capillary to prevent loss by spattering. The delivery tube is made by drawing out 6 mm. glass tubing to form a capillary of 1 to 2 mm. bore and 10 to 20 cm. long. A plug of bleached cotton wool is inserted in the wide part of the tubing; then the capillary end is heated in a microburner and drawn down to a finer tube of 0.3 to 0.5 mm. bore and about 10 cm. long. Fig. 24 shows the complete arrangement. The fine capillary delivers a stream of tiny bubbles; consequently, the solution does not spatter from the microcentrifuge tube. The gas must be started through the tube before plunging the end of the capillary into the solution. Otherwise, the solution will rise in the capillary and, when the hydrogen sulfide is admitted, a precipitate will form in the capillary and clog it. The end of the sulfide

precipitation can be easily detected by an increase in the size of the rising bubbles. At room temperature, this point is usually reached in about 3 minutes.

An adequate supply of various types of special apparatus of small capacity must be kept on hand. These are required for the liberation of volatile compounds after decomposing small quantities of solid materials or solutions with acids or alkalies. An apparatus designed for the detection of carbonate, sulfide, etc., is shown in Fig. 25. It consists of a micro-test tube of about 1 ml. capacity and can be closed with a small ground glass

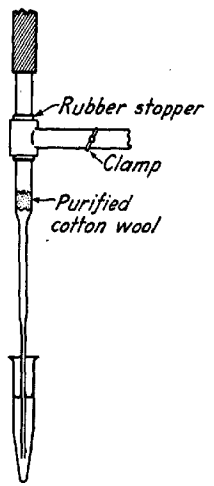


Fig. 24. Set-up for precipitating sulfides by leading in hydrogen sulfide (½ actual size)

stopper fused to a glass knob. The gas is evolved in the tube, aided if necessary by gentle warming, and is absorbed by the reagent. Since the apparatus is closed, no gas escapes, and if enough time is allowed it is absorbed quantitatively. A drop of water may replace the reagent on the knob. In this case the gas is dissolved, and the drop then may be washed onto a spot plate or into a microcrucible and treated there with the reagent. The apparatus shown in Fig. 26 is sometimes preferable, particularly when minute quantities of gas are involved. The tube is closed by a rubber stopper and the glass tube, blown into a small bulb at the lower end, may be raised or lowered at will. A change of color, or the reaction products,

may be made more distinct by filling the bulb with powdered gypsum or magnesia. In some cases, such as the test for ammonia, it may be desirable to suspend a small strip of reagent paper from a glass hook fused to the stopper (Fig. 27). The apparatus shown in Fig. 28 is used when a particular gas is to be identified in the presence of other gases. In this arrangement the stopper of the micro-test tube is a small glass funnel, and the impregnated filter paper is laid across it to absorb the gas. The impregnated filter paper permits the passage of the indifferent gases and retains only the

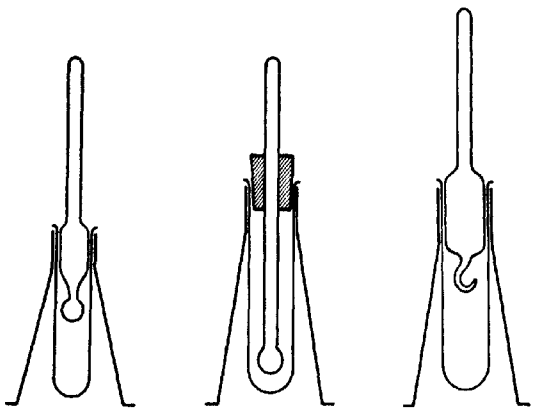


Fig. 25 Fig. 26 Fig. 27

Fig. 25. Apparatus for detecting CO_2, H_2S, etc.
(actual size)

Fig. 26. Modification of apparatus shown in Fig. 25. Especially suitable for detection of minute quantities of gas
(actual size)

Fig. 27. Apparatus for detection of gases, with provision for suspending a reagent paper
(actual size)

gas to be detected. The latter forms a non-volatile compound which can be identified by a subsequent spot test. Another useful apparatus (Fig. 29) consists of a micro-test tube containing a loosely fitting glass tube narrowed at both ends. The lower end is filled to a height of about 1 mm. with an appropriate reagent solution. If the gas evolved forms a colored product with the reagent, it can be seen easily in the capillary.

A simple hard glass tube, supported in a circular hole in an asbestos plate (Fig. 30), can be used if high temperature or ignition is required to free the

gas. The open end of the tube is covered with a small piece of reagent paper kept in place by a glass cap.

Microdistillation is sometimes required; the chromyl chloride test for chloride is an example. Very small quantities of material can be distilled

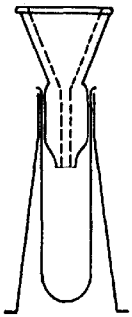


Fig. 28

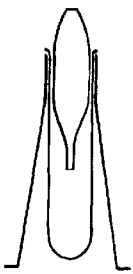


Fig. 29

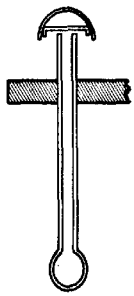


Fig. 30

Fig. 28. Apparatus for detecting a gas in the presence of indifferent gases (actual size)

Fig. 29. Apparatus for detecting a gas that forms a colored product with the reagent solution (actual size)

Fig. 30. Apparatus for detecting a gas whose release requires high temperatures (actual size)

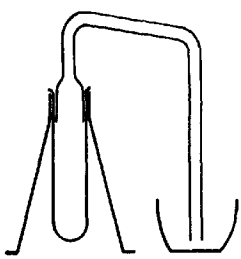


Fig. 31. Apparatus for distilling small volumes of a liquid (actual size)

in the apparatus shown in Fig. 31. A microcrucible or microcentrifuge tube can be used as the receiver.

8. *Heating of Solutions*

There are various ways of heating solutions to hasten chemical reactions in spot test analysis.

Filter paper, on which drops of liquid have been placed, can be warmed by holding it over an electric hot plate or over an asbestos plate heated by a burner. A blast of hot air is still better. More or less evaporation can be expected because of the great surface exposed by the liquid when taken up in the capillaries of the paper. When the spots are subjected to a current of warm air, it must be remembered that the material will be localized on that side of the paper nearest the heat.

Sometimes it is advantageous to heat spots on paper with avoidance of evaporation. This can be done by means of steam. A suitable apparatus is shown in Fig. 23. Its operation is self-evident. The filter paper is laid on the side arm support, kept in place with the concave side of a small watch glass, and exposed to the action of the issuing steam.

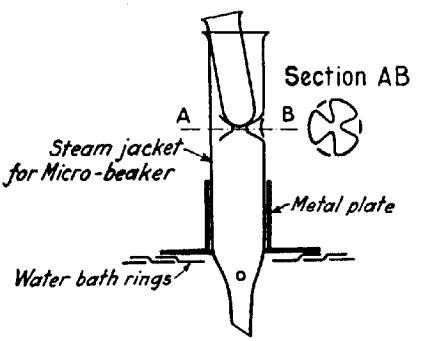


Fig. 32. Support for holding beakers, etc. on a water bath
(½ actual size)

If small volumes of liquid are to be heated with as little rapid evaporation as possible, or if a material is to be dissolved by warming with acids, alkalies, etc., small vessels of glass, porcelain, or platinum are necessary. Microcrucibles are placed on a water bath or on an asbestos plate heated by a microburner. The liquid in the crucible is stirred with a microstirrer to avoid spattering and super-heating.

A support designed to hold microcrucibles, microbeakers, or microcentrifuge tubes on the water bath is shown in Fig. 32. It consists of a hard glass tube constricted at the bottom and held in a metal ring. About 3 cm. from the bottom of the tube is a glass support for the crucible, etc., which can thus be warmed by the current of steam.

The simple arrangement shown in Fig. 33 is excellent for heating liquids in micro-test tubes. It is made by twisting together two thin aluminum

wires. The flared ends of the test tubes are easily held in place in these slings. If the wire stand, together with the test tubes, is suspended over a beaker filled with water it is easy to heat them and to carry out duplicate or parallel tests.

Whenever possible, it is best not to boil liquids; instead, solutions should be heated at water bath temperatures.

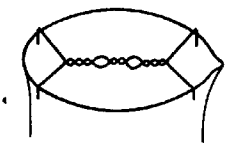


Fig. 33. Wire sling for holding test tubes, etc.
(½ actual size)

9. *Separation of Solid and Liquid Phases*

The detection of certain elements and groups of atoms can often be accomplished directly in the presence of other materials by calling on every possible means to increase the sensitivity and positiveness of chemical tests. The usual operations of separating by precipitation and filtration, which are essential in the solution of analogous problems in the classical methods of qualitative inorganic and organic analysis, sometimes cannot be used. In many cases, however, the surest procedure is still to make a division into the familiar main groups of the systematic scheme of analysis and to follow this partial separation by spot reactions to identify the individual members of the groups. This is particularly advisable in the analyses of unknown inorganic mixtures, and in the investigation of natural or artificial products. Likewise, when the analyst is asked to determine the presence or absence of a given material in a specimen, it is occasionally best, after the sample has been dissolved, to make a preliminary precipitation. The identity tests can then be carried out by means of characteristic spot reactions on the precipitate or filtrate. Furthermore, since the isolation and rendering visible of minute quantities of precipitated materials is often of great moment in spot reactions, it follows, from all the foregoing, that the separation of solid and liquid phases is a very important operation in spot test analysis, where it often has to be accomplished on a microscale.

When spot reactions are made on paper, either by bringing together two drops or by spotting a reagent paper with a drop of the test solution, any insoluble compounds formed are precipitated directly in the paper, and the unchanged constituents of the solutions undergo capillary diffusion. The latter will then be present throughout the whole spotted area, particularly

in the circular zone surrounding the precipitate. *Precipitation and filtration* are thus accomplished in the surface of the paper.* Additional spot tests can be made then on the spotted area, either on the product that has precipitated there, or on the circular zone surrounding the precipitate. Spot reactions on paper not only accomplish a direct precipitation and filtration but also make it possible to purify precipitates by washing. This can be done by placing drops of water, or of a suitable wash liquid, on the center of the spot; the concentric ring around the precipitate is thus extended by capillary diffusion. If the filtrate is of no importance for additional tests, it is better to bathe the spotted paper in an appropriate wash liquid, which can be renewed if necessary. When it is desired to wash a precipitate by repeated treatment with drops of water, each drop should be completely absorbed before the next drop is added.

It is generally better to dry the spots before washing them. This fixes the precipitate more firmly in the capillaries of the paper and there is less likelihood of its being washed away. Spots are dried best and most quickly by a blast of warm air. This localizes the material that has remained in solution and undergone capillary diffusion; the accumulation will be greatest on the side of the paper toward the blast. This is an advantage if further tests by later spot reactions are to be made on the filtrate that has been separated by capillary action.

The testing for dissolved materials, that have diffused out of a spot of precipitate, should be made by spotting laterally. It is best to place a drop of the appropriate reagent on the dry paper beyond the primary spot. The reagent will spread uniformly from the point of application, and characteristic reaction pictures will be produced at the junction of the two spots. If colored reagents are used in this manner, even slight changes in color are quite apparent.

Solids, or components of mixtures, can often be tested directly to determine their solubility in dilute acids, alkalies and the like, by spot reactions on paper. A small quantity of the pulverized sample is heaped on a strip of filter paper and spotted with 1 or 2 drops of the solvent. The action is hastened by warming in a current of heated air. Complete solution is obviously established if the sample disappears. Partial solution can be detected by applying suitable reagents near the site of the original reaction. Direct spotting of solids on paper is not limited to the determination of solubility, but can be used also if soluble colored reaction products are formed by the action of reagents and then diffused away through the capillaries of the paper. Frequently, characteristic spot reactions can be made directly on white paper through this type of filtration.

The precipitation and filtration following spot reactions on paper may not be applied to all cases, because strongly acid or alkaline solutions cannot

be used, nor is it feasible to subject reaction mixtures to prolonged and intensive heating. Neither is it possible, as a rule, to detect and isolate small quantities of colorless reaction products on paper. Consequently, other means must be employed to separate solid and liquid phases. The choice of the method is determined by the particular needs of the moment.

If considerable quantities of liquid are involved, and if the solid or precipitate is of no further interest, a portion of the liquid can be withdrawn by a pipette for examination. The fine constricted end of the pipette is closed with a wad of cotton drawn out to a point. If the suspension is sucked into the pipette, the liquid that arrives in the tube will be free of precipitate. The pipette will deliver a perfectly clear liquid if the tip is carefully washed after removing the cotton.

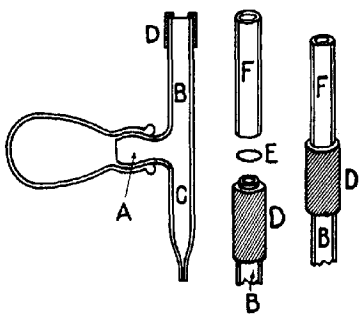


Fig. 34. Filtering pipette
(actual size)

A useful filter pipette is shown in Fig. 34. It is constructed of glass tubing (6 mm. diameter). A rubber bulb is attached to the short arm A; the arm B is ground flat; the arm C is drawn out to a fine capillary. A short piece of rubber tubing is fitted over the top of B. A disk of filter paper of the same diameter as the outside diameter of the tube is cut from a sheet of filter paper by a sharp cork borer or hand punch, and is placed on the flat ground surface of B. The tube F is placed on the paper, which is held in position by sliding the rubber tubing over it just far enough to hold it when F is removed. The filter pipette can be used either by placing a drop of the solution on the filter disk or by immersing B into the crucible, test tube, or other container holding the liquid to be filtered. The bulb is squeezed between the thumb and middle finger and the dropper point is closed with the index finger; the solution is thus allowed to pass through the paper when the bulb is released. The pipette is inverted over the spot

plate, etc., in an inclined position with the bulb uppermost when it is desired to discharge drops of the filtered liquid. The liquid in the tip is forced onto the spot plate, etc., by manipulating the bulb. The precipitate on the paper can be removed for any further treatment by simply sliding the rubber tubing D over the arm B.

Another method of filtration employs an Emich filter stick, fitted into a heavy-wall suction tube by means of a rubber stopper. The suction tube contains a micro-test tube to receive the filtrate (Fig. 35). The filter stick contains a small asbestos pad.

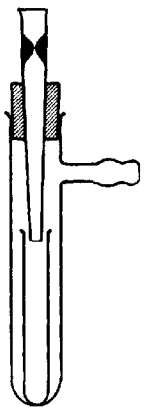


Fig. 35. Set-up for microfiltration, using filter stick and suction (actual size)

Often filtration is not the best method of separating solid and liquid phases. Sedimentation of insoluble materials by centrifuging is sometimes preferable. In addition to greater speed, this procedure has the following advantages: no retention of the mother liquor by the filtering medium; the precipitate, freed from most of its moisture, is compressed into a small volume; the structure of the solid phase (crystalline or amorphous) has no effect on the sharp separation of the phases. The receptacles for centrifuging (centrifuge tubes) can be so chosen for size that the isolation of minute quantities of precipitate or of small volumes of filtrate can be effectively accomplished.

A microcentrifuge tube is shown in Fig. 36, together with a glass support. This arrangement is useful for heating or evaporating on the water bath. A

variety of centrifuge tubes with capacities of from 0.5 to 3 ml. should be available.

Centrifuge tubes are conveniently supported on a rack consisting of a wooden block provided with 6 to 12 holes, evenly spaced and $\frac{5}{8}$ of an inch in diameter, $\frac{1}{2}$ inch deep. Microcentrifuges are now on the market. Those that are driven electrically (1500 to 3000 r.p.m.) are preferable to hand operated centrifuges. The centrifuge should be provided with a metal shield and cover to protect the operator. Dangerous vibration of the instrument is avoided by always loading the carrier equally. This is done by counter balancing the tube containing the sample by an opposing tube containing an equal weight of water or an approximately equal volume of the liquid being centrifuged. The cover of the centrifuge must not be lifted until the rotor has come to rest.

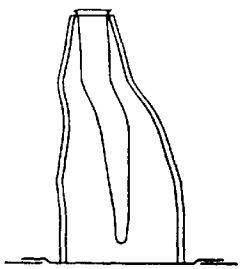


Fig. 36. Microcentrifuge tube and support
($\frac{1}{2}$ actual size)

Precipitations are usually made in conical microcentrifuge tubes. The precipitate collects at the bottom of the tube when the suspension is centrifuged. A dropper pipette can be used to remove the supernatant liquid from centrifuge tubes of about 2 ml. capacity. The liquid cannot be poured off because a large proportion will always remain in the tube. A dropper pipette suitable for this operation can be made easily from glass tubing; the dimensions are given in Fig. 6. A transfer capillary pipette is convenient for removing the mother liquid or centrifugate, particularly from smaller tubes (0.5 to 2 ml. capacity). The pipette is made of glass tubing (internal diameter about 2 mm.) which can be drawn from wider tubing. The length is 20 to 25 cm. One end is drawn to a tip with a fine opening by heating in a microflame. The correct method of transferring the liquid to the capillary pipette is made evident by Fig. 37. The centrifuge tube is held in the left hand, and the pipette slowly pushed toward the precipitate

so that the point of the capillary always remains just below the surface of the liquid. This is continued until almost the entire solution is in the pipette and the tip is about 1 mm. above the precipitate. The liquid is drained from the pipette into a clean, dry centrifuge tube.

Precipitates are washed by adding the wash solution directly to the precipitate in the centrifuge tube and stirring thoroughly either with a platinum wire or by means of a stirrer (Fig. 13). This is readily constructed from a glass rod. The suspension is then centrifuged and the clarified liquid removed with the aid of a pipette as just described. This operation may have to be repeated two or three times to insure complete washing.

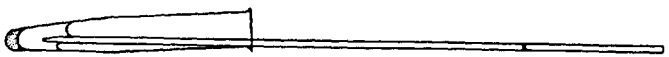


Fig. 37. Removing supernatant liquid from a centrifuge tube by means of a transfer capillary pipette
(actual size)

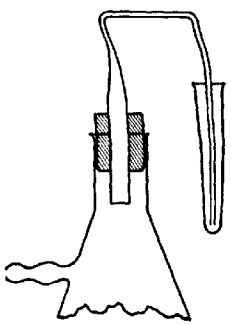


Fig. 38. Device for withdrawing liquid from a centrifuge tube by suction
(½ actual size)

Centrifuge tubes are cleaned with a feather or a small test tube brush. They are filled then with distilled water and emptied by suction using the device shown in Fig. 38. After the suction has been started and the liquid drawn out, the tube is filled several times with distilled water without removing the suction device between emptyings. Dropper pipettes are cleaned by repeated fillings with water; the bulb and tube are finally separated and both rinsed with distilled water from a wash bottle. Transfer capillary pipettes are cleaned by blowing a stream of water from a wash bottle through them.

Small quantities of a precipitate can be collected by centrifuging in a microcentrifuge tube, and thus made more visible and accessible to further treatment. This method of separating solid and liquid phases can therefore be substituted for filtration in many instances. If the problem is merely the detection of the formation of minimal quantities of precipitate, that can produce not more than a slight opalescence if the precipitate is colorless, it is frequently necessary to centrifuge for considerable periods to accomplish the separation of the colloidally dispersed solid phase. In such instances, a separation can often be accomplished quickly by shaking the suspension with an organic liquid that is not miscible with water. The surface tension is altered and the fine particles of the solid aggregate and collect as a thin film in the water-organic interface (see p. 98). This method is recommended particularly when it is necessary to detect the formation of a precipitate in a considerable volume of solution after a reagent has been added, or in a reagent solution following the addition of one drop of a test solution. The aggregation and localization by extraction or shaking out succeeds best in neutral and acid solutions. This treatment with an organic solvent is conveniently done in macro- or micro-test tubes provided with glass stoppers.

10. *Other Special Aids*

The actual detection reactions used in spot tests are carried out as a rule with drops of solutions. If the specimen is solid, the solvent should be chosen judiciously and the slightest possible excess used. This holds particularly for solvents which function by chemical action. Sometimes metals, alloys, and ores may be put into solution without resorting to a chemical solvent. An anodic solution is used instead and the anodically dissolved metal is allowed to react directly on a suitable reagent paper. This is known as the electrographic method. The principle of this procedure is that the test substance is used as anode with aluminum foil as cathode; filter paper moistened with the reagent is placed between these poles. The reagent paper is not in direct contact with the aluminum foil but is placed on a second filter paper moistened with potassium chloride or sulfate. The electrolyte facilitates the passage of the current. When the circuit is closed, the metal dissolves at the anode, and the solution reacts directly with the reagent in the paper, where it forms a typical colored stain.

A simple apparatus for such electrographic tests is shown in Fig. 39. It consists of an aluminum plate as the negative pole (Al) on which is laid first a layer of filter paper moistened with potassium chloride solution and then the reagent paper (P) moistened with water or acid. A copper plate with a copper rod soldered to it serves as the positive pole; an iron nail or

wire leads in the current. The test substance is placed between the poles or the portion to be used as cathode is given a flat surface. The current is furnished by a flashlight battery.

Alloys or minerals showing no appreciable resistance to the passage of the current may be tested with this apparatus for anodically deposited metals. Sections of metallic specimens may be tested for the location of constituents without damaging the sample, since the electrical method has the advantage that no acid reagents are required in the anodic solution of metals.

In addition to the mechanical aids already described for spot reactions, several other special devices and appliances for the preliminary operations

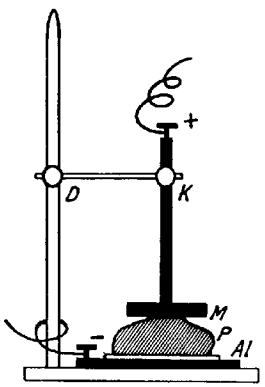


Fig. 39. Set-up for electrographic tests
(½ actual size)

should be available in the laboratory devoted to spot analysis. The most important of these are:

1. *Centrifuge:* For separating large quantities of precipitate.
2. *Microcentrifuge:* For collecting precipitates in microcentrifuge tubes and capillaries.
3. *Distillation apparatus:* The ordinary macrodistillation apparatus is usually suitable when testing residues in organic solutions. Microdistillation apparatus should be used for working up small volumes of liquids.
4. *Extraction apparatus:* A microextraction apparatus should be provided, in addition to the usual Soxhlet apparatus, for the extraction of solid materials with organic solvents.

5. *Sublimation apparatus:* A microsublimation apparatus and temperature indicator is suitable for testing the purity of organic preparations.
6. *Melting point apparatus*: For testing the purity (melting point) of organic preparations.
7. *Hot air apparatus:* For drying impregnated filter paper and for heating flecks on one side of the paper.
8. *Electric muffle (ignition) furnace with temperature regulator:* } For drying and ignition of precipitates and specimens.
9. *Electric hot plates:* / *Portable drying oven:*
10. *Analytical quartz lamp:* For detecting fluorescent reaction products; observation of photochemical reactions; comparison of samples.
11. *Microscope:* For identification of crystalline precipitates.
12. *Magnifying glasses:* For closer examination of spot tests on paper.
13. *Analytical balance:* For quantitative macrodeterminations.
14. *Microbalance:* For weighing quantities less than one milligram.
15. *Rough balance:* For approximate weighing of preparations, etc., before using the analytical macrobalance.
16. *pH meter.*
17. *Portable electrolysis apparatus:* For electrolytic deposition of metals.
18. *Colorimeter:* For colorimetric determination of dissolved colored materials.
19. *Stop watch.*

Chapter III

SURFACE AND CAPILLARY EFFECTS IN SPOT REACTIONS

The interfacial (surface) tension residing at the boundary of two phases may lead to a local accumulation of soluble and insoluble compounds. Materials which are to be identified subsequently, as well as the products of chemical reactions, are subject to this localization. Both cases are important in spot test analysis because a localized accumulation of characteristic reaction products always enhances visibility. This leads to an increase in the sensitivity and, if the accumulation is in addition selective, the test is more decisive.

There are various ways of obtaining localized accumulations of materials in spot test analysis. The most common method is to adsorb the material to be detected or a reaction product in definite zones in the capillaries of filter paper. Such local segregation frequently occurs automatically as a result of capillary action when spot reactions are made on paper. Another method is to extract a test solution, after adding a reagent if necessary, with small volumes of an organic solvent. Solution of a characteristic compound in the organic solvent may then occur or, if a compound insoluble in both media is formed, it will gather in the water-organic liquid interface. Finally, the materials being sought may be adsorbed from a dilute solution on an indifferent carrier, or they may be coprecipitated with it. The carrier can then be isolated and a special reaction used to test for the adsorbate.

The great reactivity exhibited by finely divided materials, in comparison with their activity in compact particles, is a surface effect of great importance. It is used frequently in spot test analysis. This effect is so great that sometimes slightly soluble compounds, when highly dispersed in the capillaries of paper, undergo similar reactions at practically the same rate as soluble reagents. This has the further advantage that the reaction products remain fixed at the spot where they are formed or they may produce characteristic zones. Spot test analysis makes extensive use of solid reagents, which for many reasons cannot be employed directly in macroanalysis. Papers impregnated with water-insoluble reagents can be included in this category.

If a precipitate is formed by bringing a drop of a solution onto paper impregnated with an insoluble salt, and if the reagent in the spot is not consumed entirely, the reagents will be covered superficially with the reaction product. This coating may be sufficient to protect the underlying

reagent from certain reactions. Sometimes such protective coatings are formed by very small amounts of materials which, of themselves, cannot be detected. This "protective coating effect" (see p. 85) can be put to use only in spot test analysis.

The activity due to the development of an extensive surface is responsible for the fact that sometimes reactions which are too slow or give no useful results if compact particles of the specimen are used can none the less be utilized for analytical purposes if they are carried out as spot tests on reagent papers. Reactions on reagent papers, as well as those occurring when drops of the test solution and reagent are brought together on untreated paper, sometimes directly involve the filter paper because of its adsorptive action. Consequently, many tests can be carried out with satisfactory sensitivity only as spot reactions on paper. In such cases it is justifiable to consider the paper as an active participant in the reaction.

The study and exploration of chemical changes taking place on solid surfaces or at other favorable parts of a reaction scene is the province of "topochemistry." Spot test analysis often deliberately makes use of topochemical reactions in order to increase the sensitivity and certainty of tests. The most typical of these applications of topochemical reactions are the use of papers impregnated with insoluble reagents, the direct testing of solid specimens with a solution of more suitable, more sensitive, and more specific reagents, and reactions with materials adsorbed in the capillaries of paper.

Finally, a characteristic surface effect is encountered in one of the most fundamental procedures of spot test analysis, namely the delivery of a drop of a liquid from a glass rod or a pipette. Surface tension causes a given volume of liquid to assume the geometrical form exposing the minimum surface, hence the formation of drops. This surface tension, as well as the velocity with which drops flow from capillary tubes (pipettes), depends, at constant temperature of the solvent, on the quantity and nature of the solutes. In the case of aqueous solutions, the size of the drops, within a wide range, is practically independent of the concentration. The findings are quite different with solutions in organic solvents. Even very minute quantities of dissolved materials frequently change the drop size and thereby radically alter the rate of outflow.

A. Determination of the Drop Size of Liquids by Measurement of the Wetted Surfaces

Preparation of Iodine Pictures. The common characteristic of most spot reactions is the use of a single drop of the particular test solution. Consequently, a knowledge of the drop size is essential to the measurement

of the sensitivity of tests and is also fundamental in determinations based on spot colorimetry (Chapter IX).

The exact measurement of the drop size (volume of one drop) of solutions and liquids is made by counting the drops obtained when a given volume (usually one milliliter) is allowed to flow slowly from a microburette. A simple procedure, quite satisfactory for most purposes, that can be used for aqueous and organic solutions as well as for those containing a mixture of solvents, is based on the determination of the area wetted by the complete capillary spreading of a drop of liquid placed on filter paper.

A drop of any liquid spreads uniformly in all directions through the capillaries of filter paper. A circular spot or fleck is formed; its area is proportional to the volume of the drop, providing paper of the same nature and thickness is used in comparative tests. Therefore, if a drop of liquid of known volume is placed on paper, and next to it a smaller drop of the

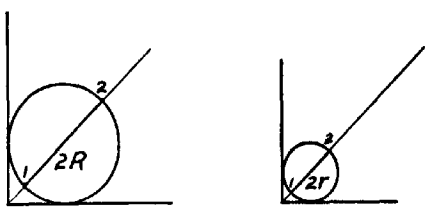


Fig. 40. Use of a right angle triangle in determining the diameter (area) of a fleck

same solution, a larger and a smaller circle will be formed (Fig. 40). If R is the radius of the spot produced by the drop of the known volume V, and r is the radius of the fleck formed by the drop of unknown volume v, then

$$R^2\pi : r^2\pi = V : v.$$
$$R^2 : r^2 = V : v.$$

It is easy to calculate v after measuring R and r, since $v = V\frac{r^2}{R^2}$, and V is known.

The radii, (R, r), of the spots being compared can be determined while the flecks remain moist, by means of the different translucence of the wet and dry paper. It is possible to make much more accurate measurements if the "iodine pictures" of the flecks are developed. The spots produced by aqueous solutions are allowed to dry in the air until there is no apparent difference, either in translucence or to the touch. The paper is then held above iodine vapor for about 30 seconds. A distinct blue will develop only in those areas that had been wetted by the drops of liquid. The iodine

volatilizes and the iodine picture will disappear on standing, but it can be regenerated by renewed exposure to iodine vapor.

Iodine pictures of non-aqueous liquids such as alcohol, acetone and even quite volatile solvents such as ether and carbon disulfide, can be produced by a somewhat different procedure. A minute quantity of iodine is dissolved in the liquid, a drop placed on filter paper and the solvent evaporated, in a current of warm air if necessary. Almost all the iodine is volatilized. If the dried paper is placed in water the spotted surfaces will exhibit a blue iodine picture, whose extent reveals the area over which the volatile solvent had spread.

The development of the iodine picture can be explained as follows: If a drop of water, or a water solution, is placed on filter paper and then allowed to dry in the air, the water is not completely removed from the wetted area. This can only be accomplished by long continued drying in warm air. The residual water, however, in conjunction with iodine vapor brings about a very slight oxidation of the cellulose of the paper. Iodide ions are produced and suffice to form blue starch-iodine with the free iodine and the starch that is present in practically all filter paper. Consequently, starch-iodine forms only in the wetted areas and so the size of the fleck will be disclosed even after the paper has "dried."

An analogous process occurs with organic solvents. These always contain traces of water which lead to a minute formation of iodide. If starch is present, the iodide, by forming polyiodide, retains traces of free iodine, even after warming. Consequently, the conditions for forming iodine-starch are also present in this case and the blue becomes clearly visible when the fleck is subsequently moistened with water.

In this connection it should be noted that dilute potassium iodide-iodine solutions containing starch are heat-stable in contrast to solutions of equal concentration that contain no starch. The blue fades on warming but reappears when the solution is cooled. Use is made of this fact in the production of iodine pictures of organic solutions. The flecks produced by volatile organic solvents cannot be measured unless the iodine picture is developed. The liquid evaporates far too rapidly to enable the operator to observe the greater translucence of the wetted areas.

Experiments

1. Single drops of water are placed next to each other on a strip of filter paper by means of a thick glass rod, a pipette, and a glass thread, respectively. Immediately after the drops have soaked in, the still moist boundaries of the spots are outlined with a lead pencil and the spots allowed to dry in the air. It is well to place the strips across a small beaker so that only the unmoistened portions touch the edges. The dried paper is placed

on an open, wide-mouth, low-form weighing bottle containing a layer of iodine crystals. After a short time, the iodine pictures of the drops of liquid, which are different in size, develop. It will be observed that the boundaries delineated in pencil do not coincide with those of the iodine pictures, but lie within them. This is particularly true of large drops. The iodine picture reveals the true extent of the capillary spreading, whereas the pencilled limits do not show the migration that slowly continues after the drop has been absorbed.

2. Several small particles of iodine are dissolved in ether, carbon bisulfide or some other volatile organic liquid. A drop of each of the solutions is placed on filter paper, the solvent allowed to evaporate, and the spot is then warmed for about 30 seconds in a blast of heated air to vaporize the excess iodine. The paper is then immersed in water, immediately removed, and the formation of the blue iodine pictures observed. These indicate quite closely the area of the flecks.

3. One milliliter of water or any desired salt solution is delivered dropwise from a micro-burette (capacity 1 to 2 ml.). The number of drops, n, is counted. The volume, v, of one drop, consequently is $\frac{1}{n}$ ml. The burette is refilled and 3 drops are discharged on filter paper, taking care that the adjacent spots do not run into each other. After the water has soaked in, the paper is dried as described in Experiment 1, and the iodine pictures are developed by the procedure given there. The area of a fleck is measured by placing the sides of a right angle wooden triangle as closely as possible on the edges of the spot and drawing, with a pencil, the tangents and also the 45° diagonal. In this way, points 1 and 2 (Fig. 40) are obtained, and consequently the diameter ($2R$) of the fleck. An estimate of the accuracy of the measurement is obtained by determining the area of the three spots produced under parallel conditions. The average of the three trials is taken as the value for R. The experiment is repeated with another series of smaller or larger drops, whose volume is to be determined. The value of v is obtained by means of the expression:

$$v = V\frac{r^2}{R^2}$$

4. A drop of water and a drop of iodine dissolved in organic liquids are placed on different varieties of filter paper by means of microburettes. The spots are dried, the iodine pictures developed by the foregoing procedure, and the wetted areas measured. The diameters of the flecks give a good idea, and a basis for the comparison of the relative absorbing powers of the papers and also furnish information as to the uniformity of single varieties of paper.

Permanent preparations of the iodine pictures of water drops can be made by marking the bounds of the iodine pictures with a pencil and then storing the papers in well-fitting Petri dishes. The iodine pictures fade almost completely on standing, but can be renewed at any time by re-exposure to iodine vapor. The limits of the restored pictures will agree with the bounds marked in pencil.

B. Capillary Spreading of Water and Dissolved Materials on Filter Paper

Purest washed filter paper consists of practically pure cellulose; the content of inorganic materials is extremely low. It owes its purity (see p. 37) to an extensive bleaching process, to repeated treatment with hydrofluoric acid and other chemicals, and to careful washing. In contrast to smooth paper, it is unsized, and since it consists of a complicated system of capillaries pressed into each other, it has a great ability to absorb liquids.

If the end of a strip of filter paper is put into a liquid, or if a drop is placed on filter paper, the liquid rises because of the suction action of the capillaries, or spreads out uniformly from all sides of the spot. The capillary spreading of the drop of liquid placed on paper is particularly important for purposes of spot test analysis because all sorts of chemical reactions can occur in this wetted area. When a drop of liquid is placed on dry paper, it forms a mound and this disappears gradually as the liquid spreads out into the interior as well as toward all sides. The typical phases of this spreading are shown schematically in cross section in Fig. 41. The rate and extent of the spreading of liquids that evaporate slowly depend on the imbibing power and the thickness of the paper.

The completion of the capillary spreading leads (as shown in Fig. 41) to a local wetting of the paper in the form of a cylinder whose height is determined by the thickness of the paper. The wetted portion is only approximately cylindrical. A perfect cylinder does not result because the porous filter paper is not completely homogeneous; furthermore, the compression of the paper is not uniform in all directions. In particular, paper that has been passed through rolls contains more fibers pointing lengthwise. As a consequence the fleck is not perfectly circular but elliptical, and its outline is jagged.

When a paper is wetted by a drop of solution, solvent as well as solute is retained by adsorption in the capillaries of the paper, each to a different degree. So far as the solvent, water for example, is concerned, it is retained in the highest proportion in the center of the spot, and from there its ratio decreases in the direction of the capillary migration (Experiment 1).

From aqueous solutions, which the analyst encounters almost exclusively, the solutes, as a rule, are adsorbed more strongly by the capillaries of the

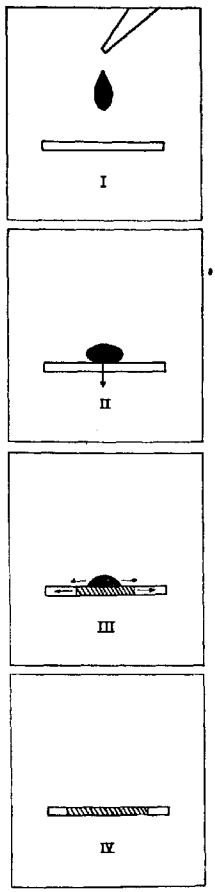


Fig. 41. Typical phases of the absorption and spreading of a drop of liquid applied to filter paper

paper than is the water, that is, to a higher per cent fraction of the effective quantity. Accordingly, if a drop of salt solution is placed on paper, a partial segregation ensues, or more correctly, a change in concentration within

the field of the spot. In the interior of the spot, the dissolved material remains behind along with part of the water, and forms a circle, surrounded by a ring of pure water. It should be noted that the distribution within the central area is not uniform. There may be a constant decrease in concentration from the center to the edge, or an accumulation along the outside of the inner circle, forming a ring whose width varies from case to case. A ring is formed only if very dilute solutions are involved, and results from the fact that at first water alone is extracted from such solutions by the paper. The solution which is spreading by capillary action is thus concentrated. Not until a definite threshold value of the concentration is reached is the solute adsorbed to a marked degree along with the water, which is continuously retained. Consequently, the inner side of the ring marks the beginning, and the outer side, the ending of the adsorption of the dissolved material. Simultaneous adsorption of water and solute can occur at the point of application of the drop if the solution is a concentrated one. In this case only a central adsorption circle can develop and always with a steady impoverishment from the center toward the outside. In isolated cases, depending on the nature and concentration of the dissolved material, the adsorption on paper may occur in two circular zones, separated by a water ring. This double ring formation is due to the sequence of events: the bulk of the solute is adsorbed in the first ring and its concentration is thus so greatly decreased that only water is adsorbed in the contiguous circular zone. The spreading solution again becomes more concentrated and finally reaches the point at which the dissolved material is again adsorbed, forming the second zone.

Different types of capillary pictures can thus be produced when drops of a solution are placed on filter paper. Their form and extent are significant for spot reactions. In considering the formation of such capillary pictures it should be remembered that the capillary spreading and adsorption of a dissolved material, like that of the solvent, occurs not only along the surface of the paper but also in its interior. Fig. 42 gives the appearance, in cross section, of the capillary picture (fleck) for thin paper and modern adsorption of the solute. The aspect of the capillary picture will be altered in characteristic fashion by thick paper, strong adsorption, and small drop size. It happens sometimes that the zone of adsorptive localization of the dissolved materials does not penetrate the entire thickness of the paper. This is shown in Fig. 43.

A general representation of typical adsorption pictures is given in Figs. 42 and 43. The extent of the adsorption and consequently the actual relative magnitudes of the central adsorption area and of the water ring, that is always formed, as well as the adsorption into the interior, depend on the size of the drop, the nature of the dissolved material, its concentra-

tion, the presence of other materials, the kind of paper, and the temperature. Consequently, wide variations may be expected. In the case of well adsorbed compounds, the central adsorption fleck is small with respect to both extent of surface and penetration, and the water ring is large. This effect, which constitutes a capillary separation, is more evident with dilute solutions than with more concentrated ones. It can be observed directly if colored solutes are involved, and with colorless compounds it can be made manifest by the employment of suitable reagents (see Experiments 2, 3).

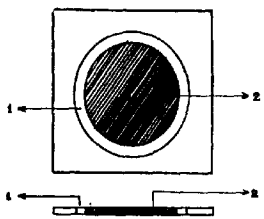


Fig. 42. Capillary picture of a slightly adsorbed material on thin filter paper
1 = water ring 2 = adsorption zone

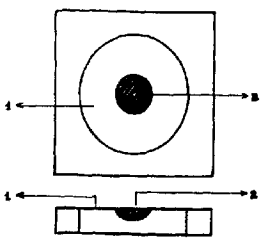


Fig. 43. Capillary picture of a highly adsorbed material on thick filter paper
1 = water ring 2 = adsorption zone

The outline and cross sections of adsorption pictures given in Figs. 42 and 43 show that most of an adsorbed material remains in a central zone of the fleck. Accordingly, any reagent used subsequently in a spot reaction must be placed in this zone. The chemical reactions occur there, and the capillary pictures given in these figures are essentially those of the flecks that remain after carrying out spot reactions. These cross sections of capillary pictures show that thin or thick filter paper cannot be used indiscriminately for spot reactions, because the capillary migration in all directions and its related adsorption differ considerably in these two media.

If the material to be detected or a characteristic adsorption product has a high adsorbability, clearer and more typical flecks will be obtained on the thicker paper. Thin paper is better, however, for compounds that are not well adsorbed, since no significant losses of material are experienced as a result of the capillary migration into the interior of the paper. In spot test analysis the proper choice of filter paper plays an especially important rôle in the search for extremely small quantities of materials, because under such conditions due regard must be given to all the factors that may raise or lower the sensitivity of the test. As a general rule, the most suitable type of paper for the spot detection of minimal quantities of material must be determined by trial.

The capillary picture of a drop of methylene blue or silver (thallium, lead) salt solution shows the adsorption of the dye stuff ion or of the metal ion (Experiments 2, 3). Salt molecules are never adsorbed from dilute solutions but only certain ions. Complex compounds or their ions can also be adsorbed on paper. Experiment 4 shows the different capillary pictures and, in part, also the different stability of the complex ions HgS_2^{--}, SbS_3^{---}, and $Ni(CN)_4^{--}$, when drops of solutions of the respective alkali salts are brought onto filter paper.

Special attention should be given to the behavior of filter paper toward aqueous colloidal suspensions (hydrosols). These owe their stability to an electrical charge residing on the dispersed particles, which are themselves tiny bits of solid. The charge is due to the adsorption of ions. Removal of the charge leads to aggregation of the particles and the formation of a precipitate. Filter paper is charged negatively when it is suspended in water. Consequently, if colloidal solutions rise or spread in paper, phenomena are observed that are connected with the sign of the charge of the sol. For instance, if a drop of a colored hydrosol is placed on filter paper, it diffuses at first in the capillaries of the paper. Sols with negatively charged particles (metal or sulfide sols, etc.) spread, and no separation of the dispersion medium and the disperse phase is observed. Positively charged hydrosols (many oxide sols, for instance) behave quite differently. The colorless dispersion medium (water) travels ahead, while the disperse phase, after slight spreading, remains fixed, forms a sharp boundary, becomes localized, and finally coagulates. The reason for the contrasting behavior of negative and positive sols is that negatively charged colloidal particles pass undisturbed through the paper capillaries which carry a like charge, whereas positively charged particles are coagulated and then clog the capillaries. Experiments 5 and 6 demonstrate the contrasting behavior when drops of several colloidal solutions are placed on filter paper.

In general, spot reactions seldom deal directly with colloidal solutions. Nevertheless, the behavior of such systems, is worth careful consideration

here because precipitation reactions invariably involve the colloidal condition as a phase of more or less stability that must be traversed. If the stoichiometric equation of a precipitation reaction is $A^+ + B^- = AB$, in which A^+, B^-, and AB are the reacting ions and insoluble product, respectively, it presents only the fate of the ionic species to the point of their union to produce the soluble individual molecule. It tells nothing about the subsequent aggregation of the soluble AB molecules into a visible phase (solid, amorphous, or crystalline). Since the transformation of the primary individual molecules, through intermediate polymerized forms up to the visible separation of a precipitate, always passes through the colloidal state of dispersion, precipitations carried out as spot reactions on paper always include the possibility of a direct intervention by the paper. Through neutralization of the charge and adsorption, the paper may bring about an aggregation of the colloidal particles and cause local precipitation of insoluble compounds in its capillaries. At the same time, the dispersion medium or solvent will continue to spread and carry along the dissolved materials. Thus slight quantities of colored solutes will be made more visible on the white surface of the paper. For this reason, when dealing with dilute solutions under circumstances in which there is particular danger that precipitates will remain colloidally dispersed, it is often better to carry out the reaction on paper, where the results will be more distinct, rather than with drops on non-porous media, or even in a test tube, where larger volumes can be used. The same improvement in visibility will also be experienced with color reactions if a colored soluble reaction product is adsorbed in distinct zones on paper.

The supplementary diffusion processes, as well as the absorbability of the reactants and the products, play a rôle when reactions are made by successively bringing a drop of the test solution and the reagent onto paper. Diffusion is the voluntary uniform distribution of dissolved materials when diluted. It is a consequence of molecular movement (osmotic pressure). Different substances have different diffusion velocities. Diffusion also occurs when a drop of a dilute solution is placed on paper and spotted directly with a drop of water or a drop of an aqueous reagent. Any soluble and reversibly adsorbed materials will be carried along by the water in the capillaries of the paper. The primary fleck spreads, the dissolved materials are distributed over a greater surface, and the result is equivalent to a dilution.

The diffusion process is somewhat different if the primary fleck of a salt solution is allowed to dry, or if a drop of water is placed on dry paper that had been impregnated with a water-soluble compound. Solution and diffusion will then cause a dilution in the center of the fleck. The dissolved material migrates farther, on the way it loses water by absorption,

and a local accumulation of material produces a ring at the edge of the fleck. As stated before, the diffusion velocity of materials differs from case to case. Consequently, with compounds that are not adsorbed by paper, or are adsorbed reversibly, the diffusion determines the extent of the capillary migration and spreading.

The capillary migration of water and dissolved materials in paper is commonly called "diffusion"; the rate of travel, the "diffusion rate." These terms are fundamentally incorrect, since the physical definition refers to diffusion solely as the penetration of like phases into each other, that is, of liquids into liquids, of gases into gases. Accordingly, diffusion, in the strict sense, occurs only in the area of the moistened portion of a fleck when water or an aqueous solution is brought into contact with it. The primary spreading of liquids through dry paper is not really diffusion. Nevertheless, the diffusion velocity of dissolved materials has a real significance in the development of capillary pictures. It is a general rule that the extent of capillary migration or the velocity of capillary migration parallels the diffusion velocity in solutions. Consequently, the diffusibility of a material, along with its adsorbability, determines the extent of its capillary migration and spreading on filter paper.

Experiments

1. A drop of a solution of iodine in ether or carbon disulfide is placed on filter paper. The solvent evaporates, leaving a brown stain of iodine. The evaporation is not uniform but proceeds from the edge of the fleck toward the middle. The deposit of the iodine shows that the solvent evaporates most rapidly at the edges of the fleck where there is most free surface. Furthermore the solvent is not distributed uniformly throughout the fleck; its proportion increases from the center toward the outside. Aqueous solutions yield the same type of evaporation pictures, but since water evaporates more slowly the phenomenon cannot be observed at once, but only after some time.

2. Single drops of 0.01 per cent solution of methylene blue are placed on thin and thick filter paper. In both instances, because of the adsorption of the dye, blue central circles surrounded by colorless water rings are obtained. The dye adsorption extends over a far greater area on the thin paper. The intensity of the colored fleck is almost equal on both sides of the thin paper, whereas, on thick paper, the color will be found solely on the side that is moistened, and water alone reaches the under side. The experiment should be repeated with more dilute methylene blue solutions and the corresponding formation of rings observed. These experiments demonstrate the effect of the kind and thickness of paper, and of dilution, on the aspect of the capillary pictures of well-adsorbed materials.

3. Single drops of 0.007 per cent $AgNO_3$ solution and of 0.01 per cent thallium carbonate solution are placed on thick absorbent paper. After the liquid has been absorbed, the spots, while still moist, are fumed with hydrogen sulfide. Black circular flecks of Ag_2S and Tl_2S, respectively, are formed, surrounded by colorless water rings. The experiment should be repeated with more dilute salt solutions, and the increased width of the water rings noted. Iodine vapor can be substituted for hydrogen sulfide in developing the flecks. It will suffice to hold the moistened surfaces over a weighing bottle containing iodine. In the latter case, the water rings will become blue, while the zones in which the Ag^+ and Tl^+ ions have been adsorbed remain colorless, because of the formation of AgI and TlI, respectively. These experiments demonstrate the central adsorption of Ag^+ and Tl^+ ions from aqueous solutions, and also the influence of concentration on the relative size of the adsorption circles and water rings.

4. Solutions of K_2HgS_2, Na_3SbS_4, and $K_2Ni(CN)_4$ are required for this set of experiments. The mercury solution is prepared by adding a solution of K_2S, drop by drop, to a solution (0.7 per cent) of $HgCl_2$ until the HgS, that appears at first, is dissolved. The antimony solution is a water solution (0.8 per cent) of Schlippe's salt ($Na_3SbS_4 \cdot 9H_2O$). The nickel solution is obtained by adding KCN drop by drop to a solution (1 per cent) of $NiSO_4$ until the original precipitate of $Ni(CN)_2$ has dissolved. The resulting solution of $K_2Ni(CN)_4$ is treated with a few drops of alcoholic dimethylglyoxime solution and any precipitate removed by filtering. Single drops of each of these solutions are placed on a spot plate and on filter paper (S. and S. 589 Black Band). The solutions on the spot plate remain unchanged, especially if rapid evaporation is prevented by covering the depressions of the spot plate with watch glasses. In contrast the flecks on the paper exhibit the following behavior:

K_2HgS_2: almost immediate formation of a black ring of HgS surrounding a colorless central circle.

Na_3SbS_4: a uniform deposition of Sb_2S_5 begins after a few minutes over the whole area of the fleck and becomes continuously more intense.

$K_2Ni(CN)_4$: after several minutes a small pink band begins to form at the extreme edge of the fleck; proceeding from this a pink coloration gradually develops toward the interior; only after several hours, is the whole fleck bright red.

The diverse behavior of the three complex salt solutions on paper can be explained as follows: When a solution of K_2HgS_2 is brought onto paper, the compound decomposes

$$K_2HgS_2 = K_2S + HgS$$

because of the strong adsorption of K_2S in the center of the fleck. The

presence of K_2S there can be established by spot testing with a solution of $CdCl_2$; yellow CdS is formed.

If a drop of a dilute solution of Na_3SbS_4 is placed on paper no capillary separation into Na_2S and Sb_2S_5 occurs, but the Schlippe's salt, being finely divided, undergoes oxidation (of the Na_2S), and then Sb_2S_5 mixed with oxysulfide deposits.

The great stability of the $Ni(CN)_4^{--}$ ion is responsible for the fact that *the complex-forming cyanide ions of the* $K_2Ni(CN)_4$ *solution oxidize with* extreme slowness when exposed to the air. The $Ni(CN)_2$ produced reacts with the dimethylglyoxime present and forms red nickel dimethylglyoxime.

5. Two test tubes are charged with 10 ml. of 0.001 per cent lead acetate solution. One sample is treated with hydrogen sulfide water, the other with an equal volume of colorless ammonium sulfide solution. A brown colloidal suspension of lead sulfide will be obtained in both. A drop of the colloidal dispersion is transferred from each of the test tubes to thick filter paper (S and S. 601). It will be observed that the alkaline sol coagulates in the center of the fleck and forms a brown-black circle (PbS) surrounded by a colorless water ring. No adsorptive accumulation of PbS will be observed with the acid sol; the entire fleck is uniformly wetted with the sol, and no color will be seen because of the high dilution. The experiments show that the alkaline sulfide sol, because of its positive charge, is coagulated by the negatively charged paper, whereas the acid (negatively charged) sol passes unchanged along the capillaries of the paper. The coagulation renders the minute quantity of lead sulfide contained in one drop of the alkaline sol easily visible, while the same quantity of lead sulfide in the acid sol cannot be seen because it is distributed over the entire fleck.

6. Single drops of new and of used mineral oil are placed on thin paper. The oil slowly seeps away through the capillaries of the paper. A perfectly homogeneous grease spot is left in the first case, while the used oil, which is a colloidal suspension of carbon particles, deposits the impurity in the center of the fleck. These experiments, which can be utilized also for differentiating between new (clean) and used (dirty) lubricating oil, show that the phases of non-aqueous sols can likewise be separated by capillary action of the paper.

7. A drop of 0.01 per cent eosin solution is placed on filter paper. After it has soaked in, a drop of water is brought on the center of the fleck. The eosin is driven outward through the capillaries of the paper, leaving behind a more dilute and therefore lighter area in the center of the spot. This experiment shows that subsequent spotting with water can cause the desorption of loosely adsorbed materials which are then carried away by capillary migration.

8. A drop of 0.1 per cent aqueous methylene blue solution is placed on

filter paper, allowed to soak in, and then treated with a drop of water. In contrast to experiment 7, the fleck remains unchanged. This experiment shows that strongly adsorbed materials cannot be made to move on by the addition of water.

Experiments 7 and 8 are extreme cases of the capillary eviction or retention of materials adsorbed in the capillaries of paper. The behavior of inorganic and organic compounds adsorbed on paper may be like those demonstrated above, or they may lie between these extremes. The nature of the compound will determine its behavior under these conditions.

C. Chemical Reactions and Capillary Separations on Filter Paper

The most important processes leading to the development of the capillary picture after placing a drop of liquid (water, true and colloidal solutions) on paper were described in the foregoing section. They are: (1) capillary migration of water and the dissolved or suspended materials along the surface and into the interior of the paper; (2) diverse retention by adsorption of water and its dissolved or suspended substances; (3) dilution and spreading resulting from the diffusion and carrying along of materials dispersed or adsorbed in the paper. All these processes are of importance if chemical changes are carried out as spot reactions by uniting drops of test solution and reagent on paper. It must always be remembered that the reactants never remain entirely at the place where they are applied, but that displacements ensue because of capillary migration, diffusion, and adsorption. This is also true of soluble and insoluble reaction products which are formed during the movement of the reactants. A movement of insoluble reaction products occurs because they are subject to capillary migration not only in their primary stages of colloidal dispersion, but also because even coarsely dispersed particles can be carried along mechanically by water that is spreading through capillaries. The result of the movement of material, due to the operation of various forces, is easily seen when the reaction products are colored; they spread out in the plane of the paper and form colored zones of varying intensity.

Another method of carrying out spot reactions merits particular attention. In this, a drop of the test solution is placed on paper impregnated with a reagent which is not soluble in water. This circumstance is of great advantage as the reagent is not carried away by diffusion. Consequently, the spreading drop and the materials dissolved in it encounter adequate quantities of the finely divided and therefore active reagent throughout the entire area over which the drop spreads. As a rule, more uniformly characteristic flecks, limited to a smaller area, are obtained in this manner rather than if drops of the test solution and reagent are brought together on

plain paper. Consequently, whenever possible, and especially when seeking minimal quantities of materials, preference should be given to the use of reagent papers impregnated with insoluble compounds (Experiment 1).

Stability and the highest possible uniformity of distribution of the reagent should be kept in mind when reagent papers are prepared. However, the capillary action of the paper often limits the extent of approaching these goals. Uniform distribution of a material in paper is best attained if the paper is soaked with a solution of the solid, and the solvent then allowed to evaporate. The solid remains in and on the capillaries of the paper. Compounds which are coarsely crystalline and non-hygroscopic produce, as a rule, less stable reagent papers than microcrystalline, amorphous, and hygroscopic compounds. The reason is that recrystallization, that is, the development of coarser crystals destroys the fine state of subdivision, and the solid may then fall off the dried paper more readily. Another factor working against a perfectly uniform distribution of a reagent in paper is the fact that when a moist reagent paper is dried, the dissolved material enters the capillaries along with the evaporating solvent. Consequently, there is an accumulation on those parts of the paper where the evaporation proceeds most rapidly, namely, on the surface and the edges of the paper. If, for instance, moist impregnated paper is dried in a blast of heated air, the side of the paper turned toward the warm air will contain more reagent than the other side (Experiment 2). This one-sided accumulation sometimes is advantageous. However, if a uniform distribution is desired, the paper should be allowed to dry slowly in the air or in a drying closet.

One factor which must always be kept in mind when preparing reagent papers is the stability of the reagent used for the impregnation. Reagent papers that will keep well can be prepared only from absolutely stable and pure compounds. Any tendency of the reagent, or of the impurities in it, to decompose will be greatly enhanced because of the high dispersion in the capillaries of the paper. Consequently, the decomposition will proceed much more rapidly than when the material is in compact masses, or pulverized, or dissolved.

It has been pointed out that a dissolved material, distributed in the capillaries of paper, is carried toward the scene of the evaporation when the solvent is removed, and thus is localized when heat is applied on one side only. This effect is not only important in the preparation of reagent papers; it can be utilized to increase the sensitivity of spot reactions. The increased sensitivity secured by warming the fleck is easily understood if the capillary phenomena are reviewed once more. After a drop of the test solution is placed on paper a part of any slightly adsorbed material always passes from the surface into the interior of the paper. The reaction occurs

on adding a drop of the reagent but, because of the diffusion, a further displacement and dilution takes place at the same time. This transfer is lateral as well as vertical in the paper. Consequently, all of the material enters into the reaction, at least to the extent permitted by the law of mass action, but only that fraction of the reaction product which is on the surface of the paper is clearly visible. If, however, after placing a drop of the test solution on the paper, it is heated on the side where the drop was applied, the evaporation of the solvent leads to an accumulation of the dissolved material on this side, and the reagent thus subsequently brought on the scene gives a more distinct reaction picture. The consequence is a considerable increase of sensitivity as compared with that obtained by the same procedure, but without warming (Experiments 3 and 4).

A discussion of the capillary pictures and the consideration of chemical reactions on filter paper has thus far taken into account only the behavior of solutions containing one solute. If a solution of several materials is involved, as is usually the case in chemical analysis, each species (ionic or molecular) behaves, with respect to the capillary picture, practically as though it were present alone. Consequently, adsorbable materials usually produce overlapping adsorption zones in the central circle and form a common outer water ring. Separate adsorption zones may be expected only when there are considerable differences not only in the adsorbability but also in the concentrations of the co-solutes. Differences in the adsorbability, which might lead to a sharp capillary separation of co-solutes in different zones of a pure filter paper, are seldom encountered among inorganic materials. Such capillary separations can never be counted on when dealing with unknown mixtures. The conditions are better among organic compounds, especially dyes. The components of mixtures of dyes can often be detected directly by spotting on filter paper where they form zones of different colors. This is an example of "capillary analysis," that is, the formation of typical capillary pictures (Experiment 5).

The overlapping of adsorption zones, produced by placing a drop of a solution of several materials on filter paper, usually makes it impossible or unsatisfactory to spot test for materials separated by capillarity even though suitable reagents are available. On the other hand, the capillary and reaction picture obtained by placing a drop of test solution on paper impregnated with certain reagents may be of practical analytical significance. Characteristic changes produced by a localized reaction can often be observed in solutions containing one or more solutes (Experiments 6 to 8). If the paper contains a reagent which can react with several co-solutes, then, in case insoluble products are formed, fractional precipitation occurs. The depositions follow in concentric zones, of which the innermost corresponds to the compound with the smallest solubility product. Such

fractional precipitations can be observed directly in only rare instances if carried out in test tubes. Capillary separations sometimes may even be utilized analytically by taking advantage of the capillary migration in paper and the retention of reaction products.

The formation and fixing of precipitates in distinct zones of the paper, accompanied by capillary migration of the liquid phase, is equivalent to a precipitation and filtration in the plane of the paper. Accordingly, it is sometimes possible to achieve two tests with one drop of a test solution. The spot reaction produces a precipitate, and the surrounding zone, corresponding to the filtrate, gives a second precipitate on treatment with a second reagent. If the filtrate has a characteristic color it can be seen directly as a colored zone around the precipitate fleck. The filtrate, or the materials dissolved in it, can be transported further out by additional drops of water; the precipitate fleck is thus subjected to a washing process (Experiments 9, 10).

As a rule, the special reagent is used in excess in reactions which are of analytical value. A characteristic phenomenon is then encountered if very small quantities of materials are to be detected by spot reactions on paper. The reaction product is never fixed as a circular spot, but will be found in a more or less sharply defined ring zone. The formation of this ring is due to two causes. When a drop of a dilute solution is put on paper, water is retained in the center of the fleck and the solution, which is travelling outward from this region by capillarity, becomes more and more concentrated until the reaction product formed by the reagent becomes visible. Furthermore, during precipitations, particles of precipitate, up to a certain size, but particularly in colloidal states of dispersion, are carried along by the moving liquid. The movement stops only when these finer particles agglomerate. This ring formation, which is very typical in tests for small quantities of substances, is observed both with soluble and insoluble reaction products. It occurs when drops of test solution and reagent are brought together on plain paper, as well as when a drop of test solution is placed on a reagent paper.

If chemical reactions are carried out on filter paper there is sometimes a change in the reactivity of materials adsorbed on the capillaries of the paper, or precipitated there in a state of fine division. The change in activity may be manifested by the fact that certain reactions occur more quickly or more slowly than when the material is in a more compact form. Heightened reactivity, for instance solubility in acids, is always the consequence of a higher degree of dispersion and therefore of the development of a greater reacting surface. Decreased activity is brought about by a superficial coating with resistant materials, or because the attack by reagents is hindered by the adsorption of the material on paper. Such phenomena,

which can alter the reaction picture in spot tests, will be discussed later in greater detail.

Summarizing, it is now clear that a spot reaction on paper which, at first glance, appears to be a very simple process, in reality involves a rather complicated array of coincident and successive chemical and physical phenomena. These are affected by the concentrations of the reacting materials, the order in which they are brought on the paper, the use of solid reagents, the chemical and physical properties of the reaction products, the type of paper, the accompanying materials, and the temperature. The sum total of all the factors and processes determines the form and aspect of the particular spot picture and also the sensitivity and certainty of the spot test.

Experiments

1. Single drops of a weakly ammoniacal nickel sulfate solution (0.001 per cent) are added to single drops of 1 per cent alcohol solution of dimethylglyoxime on a spot plate and on filter paper. For comparison, a drop of the nickel sulfate solution is placed on dimethylglyoxime paper. (This is prepared by impregnating filter paper with alcoholic dimethylgyoxime solution and drying.) The nickel-dimethylglyoxime reaction is not visible on the spot plate nor on the untreated paper, whereas a distinct reaction is obtained on the impregnated paper. The experiment illustrates the increased sensitivity of a spot test procurable by using a filter paper impregnated with an insoluble reagent.

2. Single drops of potassium bichromate solution (1 per cent) are placed on thin and thick filter paper. After the drops have soaked in, the broad side of the paper is held in a blast of heated air. When completely dry, the paper is cut through the middle of the fleck and the intensity of the color is compared by placing the front and rear sides in juxtaposition. The experiment is repeated, but the paper is now dried in the air by fastening it with a pin to a dangling string. The experiments teach that intensive heating of one side of a paper moistened with a salt solution results in an accumulation of the solute on that side where evaporation takes place more rapidly.

3. A drop of cadmium sulfate solution (1 per cent) is allowed to soak into thick filter paper and then dried in a current of heated air. Another drop of the same solution is then placed next to the dried spot and both are then held over a vessel containing warm ammonium sulfide. Yellow cadmium sulfide will form on both flecks. If these are now examined only a slight difference will be observed. If, however, the paper is plunged into water it will become quite evident that more cadmium sulfide has deposited on the surface of the paper from the dried fleck than from the other. The experi-

ments again demonstrate that if a drop of salt solution is placed on paper and then subjected to one-sided heating, the material accumulates on the side of the more rapid evaporation. This localization can lead to an increase in sensitivity of subsequent spot reactions. However, the transparency of moist flecks must always be considered. If flecks are warmed, care must be taken that the heating takes place on the side where the drop was applied.

4. A drop of potassium chromate solution (0.005 per cent) is absorbed on thick filter paper and the spot is outlined in pencil. The fleck is then dried in a blast of warm air. A second drop of the chromate solution is placed next to the dried fleck and allowed to soak in. Both are then spotted with 0.1 *N* silver nitrate solution. A circular deposit of red silver chromate will be observed only on the fleck that was dried before applying the silver nitrate solution. This experiment also demonstrates the favorable effect on the sensitivity of a spot test secured by unilateral heating of the fleck.

5. Aqueous solutions of methylene blue (0.0002 per cent) and eosin (0.01 per cent) are prepared. Thirty ml. of eosin solution are mixed with 20 ml. of water and a second 30 ml. of eosin are mixed with 20 ml. of the methylene blue solution. These two solutions look exactly alike. If single drops of the diluted pure solution of eosin and of the mixed dye solution are placed next to each other on thick filter paper (S. and S. 601) the following will be observed: the methylene blue is adsorbed from the mixture and a circular blue spot with a blue ring result, while the eosin travels farther and produces a pink ring around the central circle. The experiment illustrates the capillary separation of a basic dye (methylene blue) from an acid dye (eosin). The underlying reason is the difference in the intensity with which they are adsorbed on filter paper.

6. A drop of 0.1 *N* hydrochloric acid is placed on Congo paper. A blue fleck forms immediately on the red paper, since the dye, Congo red, is an indicator and shows this color change in the pH range 3.0 to 5.2. The hydrochloric acid is diluted one hundred fold, and a drop of this approximately 0.001 *N* solution is placed on the Congo red paper. This time no blue fleck appears, but the center of the spot contains a round red zone surrounded by a blue ring, and this in turn, is encircled by a second red ring.

The spot picture of the local indicator change is produced about as follows: When a drop of the very dilute acid is placed on the red paper, at first practically nothing but water is held back by the capillaries of the paper (red central area); the solution that is travelling outward is thus concentrated and when the H^+ ion concentration becomes great enough the indicator changes to blue. This condition is maintained as long as

sufficient H^+ ions are still present (blue ring). The remaining water which continues to travel outward, then merely moistens the indicator paper (red ring).

This experiment succeeds with other indicator papers, such as litmus and dimethyl yellow. It demonstrates the localization and activity of an ionic species in distinct zones of a reagent paper.

7. A neutral solution of nickel sulfate is treated with dimethylglyoxime (DH_2) and the red nickel dimethylglyoxime is filtered off. Since the precipitation from neutral solution is incomplete, the filtrate is an equilibrium solution containing all the materials involved in the reaction:

$$Ni^{++} + 2DH_2 \rightleftharpoons Ni(DH)_2 + 2H^+$$

Any withdrawal of H^+ ions from this equilibrium solution will lead to precipitation of nickel dimethylglyoxime. This can be demonstrated by adding basic materials such as NaOH, $CaCO_3$, etc.

If single drops of the equilibrium solution are placed on both thin and thick filter paper, the following will be observed. On the thin paper, after about a minute, a narrow red ring, whose intensity is the same on both sides of the paper, will form around a colorless central area. On the thick paper, however, a ring is formed at first only on the side where the drop was applied, and a red circular fleck forms immediately on the under side of the paper.

These diverse spot pictures on thick and thin filter paper result from the fact that filter paper adsorbs H^+ ions and thus disturbs the equilibrium. Accordingly, red nickel dimethylglyoxime is deposited in zones that adjoin the zones of H^+ ion adsorption. The H^+ ions can penetrate thin paper completely in the direction of the application of the drop; their capillary migration is limited only toward the sides. Consequently, the central colorless area is the zone of H^+ ion adsorption, and adjoining this is a circular deposit of nickel dimethylglyoxime. The H^+ ions do not completely penetrate thick paper; only the solvent, water, with the other dissolved materials, reaches the under side. Accordingly, the rear of the paper is impoverished with respect to H^+ ions, and nickel dimethylglyoxime forms a circular deposit there. The zones of H^+ ion adsorption can be made visible by placing a drop of bromthymol blue indicator solution close to the fleck. The colorless central area on the thin paper will turn yellow on both sides of the paper, while only the place of application becomes yellow on the thick paper.

The experiment can be varied by placing a drop of the equilibrium solution on blue litmus paper. At first a red central area is formed; accordingly, it is the zone of H^+ ion adsorption. A distinct precipitation of red nickel dimethylglyoxime forms a ring around the circumference of this

circle, but remains within its bounds. This is in agreement with the familiar fact that nickel dimethylglyoxime can also be precipitated from weakly acid solutions.

The experiments demonstrate the disturbance of the chemical equilibrium by adsorption of H^+ ions and the decrease of the H^+ ion concentration from the center of the fleck toward the sides and the interior of the paper.

8. A drop of dilute neutral lead acetate solution is allowed to soak into neutral (violet) litmus paper. A blue ring forms at a slight distance from the center of the fleck, and also a red ring separated from the first by a violet ring. Consequently, the indicator paper shows an alkaline (blue), neutral (violet), and acid (red) zone.

The formation of these zones derives from the fact that an aqueous solution of lead acetate is partially hydrolyzed:

$$Pb(CH_3 \cdot COO)_2 + 2H_2O \rightleftharpoons Pb(OH)_2 + 2CH_3 \cdot COOH$$

The colloidally dispersed lead hydroxide is adsorbed more strongly and more quickly than the acetic acid. Consequently, the litmus turns blue in the region where the basic lead hydroxide is adsorbed, whereas the indicator change produced by the acetic acid becomes visible in a separate zone only after a certain H^+ ion concentration has been reached. The experiment demonstrates the localization and reactions of the hydrolysis products of a salt in separate zones of an indicator paper.

9. Potassium chromate solution (20 per cent) and potassium permanganate solution (0.3 per cent) are prepared. Portions (2.5 ml.) of the latter are mixed with 47.5 ml. of water, and with 47.5 ml. of the chromate solution respectively. Single drops of the diluted permanganate solutions, of the chromate-permanganate mixture, and of the original chromate solution are placed on thick filter paper (S. and S. 601). The flecks should not overlap. Several minutes after the drops have been absorbed the following will be seen: The spot of pure potassium chromate solution is uniformly yellow; a faint light brown spot appears on the fleck of the pure permanganate solution; a light brown spot likewise forms on the fleck of the $KMnO_4$ $-K_2CrO_4$ mixture, but it is surrounded by a yellow ring. Of the two brown flecks, that produced by the mixed solution is definitely more intense than that coming from the pure permanganate solution.

The light brown central flecks are formed because the permanganate oxidizes the cellulose of the paper and is reduced to manganese dioxide. This is retained in a fine state of division in the capillaries of the paper. The pure chromate solution, over the period of observation, exerts no visible action on the paper, and when mixed with permanganate therefore passes outward through the capillaries of the paper and may be easily recognized because it forms a yellow surrounding zone. Small quantities of perman-

ganate mixed with a great deal of chromate can be detected much more rapidly and with greater certainty by this reaction of the permanganate with paper, and capillary migration of the chromate, than by the usual chemical methods. This procedure permits the detection of 8 γ $KMnO_4$ in the presence of 10000 γ K_2CrO_4. It is therefore the best method for detecting small amounts of MnO_4^- ions in the presence of CrO_4^{--} ions.

It was noted that the MnO_2 spot formed by the mixed solution is more intense than that produced by the pure permanganate solution. The explanation is that not only the permanganate but also the chromate of the mixed solution oxidizes the cellulose. Consequently, in addition to MnO_2, brown chromic chromate, $Cr_2(CrO_4)_3$, is formed. Chromate normally attacks paper quite slowly, but the reaction is induced by the permanganate attack on the cellulose.

The experiment illustrates a capillary separation of two co-solutes by chemical reaction of one of them with paper.

10. Filter paper (S. and S. 589) is bathed in ferric chloride solution (0.5 per cent). After the excess solution has been drained off, the paper is dried in a blast of heated air. One drop of a solution containing potassium ferrocyanide and potassium thiocyanate [1 ml. KCNS (15 per cent) + 1 ml. $K_4Fe(CN)_6$ (8 per cent) made up to 100 ml. with water] is placed on the ferric chloride paper. The following will be seen: The ferrocyanide reacts with the ferric chloride in the paper and forms insoluble Prussian blue; this remains in the center of the fleck as a round dark blue zone. The thiocyanate travels outward and produces a red ring of ferric thiocyanate around the blue zone. The red ring can be moved outward by adding a drop of water. The experiment should be repeated with a more dilute test solution. The experiment is an example of a precipitation and filtration in the plane of the paper.

D. Protective Layer Effect

The chemical reactions of solid materials, which normally proceed rapidly and smoothly, can be hindered if their exposed reactive surfaces are coated with a layer or film of resistant material. Such resistant protective layers can sometimes be produced on materials imbedded in the capillaries of paper. It is then possible to use spot reactions to detect materials capable of forming a protective layer. Therefore, the basis of the detection of a protective layer effect in spot analysis is: Paper impregnated with certain insoluble compounds is treated with a drop of a solution containing materials which, of themselves or by reaction with the material in the paper, can form a protective layer. The spot is then treated with a reagent which affects the unprotected compound only,

and therefore leaves unchanged the portions of the fleck covered by the protective coating.

Two conditions must be met if a protective layer effect is to be used for analytical purposes. First, the compound to be protected must receive a coating that is as coherent and adherent as possible. Secondly, it must be easy to observe the differences in the chemical behavior of the material forming the protective layer and of the protected compound. Consequently, reactions proceeding with production of distinct color changes are the only ones suitable for distinguishing a protective layer effect.

Theoretically the complete coating of a material with only a few molecular layers of the resistant compound should be enough to produce a protective layer. In practice, much thicker layers can be laid down on impregnated papers, but these coatings are never perfectly coherent. The imperfections arise from the fact that the interspersion and distribution of the solid material through the capillaries of the paper always leave gaps and spongy spots. The reagents seep through these, reach the interior of the coating, and react there. For this reason only a partial coating is usually obtained, but nevertheless, this sometimes leads to such marked differences in the reaction rates of the protected and unprotected portions of the paper that very sensitive tests become possible.

The best protective layer effects are obtained when the protective layer is produced by direct action with a compound imbedded in the capillaries of the paper; the coating then fits as closely as possible. Indifferent (unreactive) substances can also form protective layers. Those with a salve-like consistency exhibit good protective layer effects because they form a film over the surface of solids. On the other hand, good protection usually cannot be furnished by compounds which crystallize nicely, for, as a rule, they do not adhere well.

Experiments

1. Thallium sulfide and lead sulfide papers are prepared by soaking strips of filter paper in solutions of the respective nitrates. The strips are then exposed to the fumes of ammonium sulfide (gradual blackening, because of sulfide formation), washed briefly, and dried. Single drops of a 2 per cent solution of iodine in ether are placed on strips of the sulfide papers and the flecks held for several seconds in a stream of heated air. The finely divided black sulfides are transformed into the corresponding yellow iodides (see page 91). The spot picture on the thallium sulfide paper is especially interesting. In contrast to the lead sulfide paper, on which a yellow spot of iodide appears against a black background, a brown-black central circle is formed. This is surrounded by a narrow bright yellow ring (TII) which adjoins the black surface of the unaltered

thallium sulfide paper. The yellow band of thallous iodide is very characteristic of this fleck picture. It becomes still sharper and a little wider if the heating period is somewhat lengthened. The dark brown central circle enclosed by yellow thallous iodide is by no means unchanged thallous sulfide, as might easily be assumed from its appearance, but it consists of thallium polyiodide (TlI_3). This can be proved by placing the fleck in a potassium iodide solution (5 per cent) or by warming it in a blast of heated air for 2 or 3 minutes. The iodine of the polyiodide is thus dissolved or volatilized, the dark central circle disappears, and a homogeneous yellow fleck of thallous iodide, on a dark background, is left. (Prolonged action of the potassium iodide solution on the thallous sulfide paper slowly causes conversion into thallous iodide.) This particular spot picture on thallium sulfide paper arises from the fact that the iodine concentration is highest at the point of application of the drop. Consequently, the possibility is presented there for the formation of the thallium compound richest in iodine, namely, the polyiodide; whereas the iodine that has traveled toward the sides is only sufficient to produce the compound containing the least iodine, namely, thallous iodide. Therefore, the spot reaction shows quite distinctly the details of the action of iodine on thallous sulfide.

The iodine solution is now progressively diluted with carbon disulfide, until no formation of iodide can be seen directly when a drop is placed on lead sulfide or thallous sulfide paper. The strips of paper spotted with the diluted iodine solution are then dipped into 3 per cent hydrogen peroxide solution. The strips will be quickly and completely decolorized, except the spotted areas, which remain black to brown. The hydrogen peroxide oxidizes the sulfides to colorless insoluble lead sulfate and colorless soluble thallium sulfate, respectively, and consequently the color of the reagent papers is discharged. The spotted areas, however, where lead iodide or thallous iodide have been formed in invisible minute quantities, become brown because they still contain the respective sulfides. The slight quantity of iodide formed by direct reaction suffices to protect the underlying sulfides against oxidation by the hydrogen peroxide.

2. Filter paper is soaked in a 1 per cent solution of dimethylglyoxime and dried in a blast of heated air. Several strips of the paper are placed in ammoniacal 2 *N* nickel nitrate solution and red nickel dimethylglyoxime is thus deposited in the capillaries of the paper. This reagent paper is washed with water, then with alcohol, and dried in a current of hot air. It is stable for several weeks. On aging, the nickel dimethylglyoxime recrystallizes, part of it becoming detached from the paper, whose activity is thus lessened.

A drop of neutral solution of palladium chloride (approximately 0.01 per

cent) is brought on a strip of the red reagent paper, allowed to dry, and then placed in dilute hydrochloric acid. The color of the paper is discharged instantly, except on the site of the spot, where a red fleck is left. For comparison, paper impregnated with dimethylglyoxime alone is spotted with the same palladium chloride solution. When this test strip is plunged into dilute hydrochloric acid a yellow spot of acid-insoluble palladium dimethylglyoxime remains. The spot tests on both reagent papers should be repeated with progressive dilutions of the palladium chloride solution. It will be found that the diluted solutions, which are so weak that they no longer reveal their palladium content when placed on plain dimethylglyoxime paper or with other reagents, will still leave very distinct red flecks on nickel dimethylglyoxime paper when this is treated subsequently with acid.

This method for detecting palladium will show as little as 0.05γ of palladium in one drop. The test is strictly specific since, under the conditions of the experiment, no other metals react; neither do they decrease the sensitivity of this test for palladium.

The high sensitivity of this test is due to a protective layer effect. When a drop of the palladium solution is placed on paper impregnated with nickel dimethylglyoxime, the acid-soluble red nickel salt is converted into the acid-insoluble yellow palladium salt. This transformation occurs directly on the surface of the nickel salt, which therefore is coated with the palladium compound and protected against the subsequent attack by the acid. Quantities of palladium so small that they can no longer be detected by the formation of yellow palladium dimethylglyoxime are still capable of accomplishing this protective layer effect.

A trial with nickel dimethylglyoxime suspended in water and palladium chloride solution will demonstrate that this protective layer effect is distinctly visible only when carried out as a spot reaction on paper.

3. Single drops of a 1 per cent solution of paraffin or of a fat in very pure benzene are placed on strips of dimethylglyoxime or thallium sulfide paper (see Experiment 1). The solvent is then allowed to evaporate spontaneously or in a blast of heated air. The strips, which show not the slightest change, are then placed in dilute sulfuric acid. The red nickel dimethylglyoxime, or the black thallium sulfide, is dissolved away and the untreated parts of the reagent papers are decolorized almost immediately. Red or brown flecks, respectively, are left on the areas where the drops of test solutions were applied. As soon as the unprotected parts of the paper are decolorized the strips are placed in a dish containing water. This prevents the attack by acid on the protected areas, which would otherwise be slowly affected.

The resistance of the fleck to attack by sulfuric acid is due to a thin film

of paraffin or fat that is left when the solution in benzene evaporates. It protectively coats the surface of the nickel dimethylglyoxime or thallium sulfide. A trial will show that this protective effect can still be accomplished with the fat solution even after it has been diluted with ten times its volume of benzene. Larger quantities of fat can be detected by placing a drop of the solution on thin tissue paper or cigarette paper. A grease spot will be left after the solvent has evaporated and stands out from the unchanged paper because of its translucence. A comparison will show that the test based on protective layer effect is considerably more sensitive than the "grease spot test" just described.

An excellent reagent paper for detecting a protective layer effect is prepared by soaking filter paper in mercurous nitrate solution and then placing it in dilute hydrochloric acid. The paper is washed and dried; it contains insoluble mercurous chloride (calomel). If placed in *very* dilute ammonia water the paper gradually turns gray because of the reaction:

$$Hg_2Cl_2 + 2NH_3 = Hg + HgNH_2Cl + NH_4Cl$$

If a drop of a fat solution is placed on this calomel paper, the solvent allowed to evaporate, and the paper then bathed in very dilute ammonia, the protected area will turn gray very much more slowly than the rest of the paper.

E. The Use of Capillary Dispersion for the Recognition of Reactions Difficult to See

Solids precipitated in the capillaries of porous papers are finely divided and this development of an extensive surface is of considerable analytical importance. The surface, which is so much greater than that obtained by normal precipitation in a test tube, comes into being particularly from the fact that the solid, in so far as it does not form a more or less coherent solid film on the surface of the paper, is dispersed throughout the innumerable capillaries of the paper. Consequently, there is no aggregation into compact coarser clumps. The free surface of solids is very important in the chemical reactions of such materials because it determines, in part, the reaction velocity. This statement demands comment. The form of the solid product has practically no effect on the reaction velocity of the dissolved portions which are in equilibrium with the solid phase, but a certain fraction of the solid must have entered solution before a reaction can occur there. The speed of the passage of the solid material into the soluble phase, that is, the "physical" rate of solution, is, however, a function of the free surface; it rises and falls with it. On the other hand, there are reactions (particularly of very difficultly soluble materials) which may

occur directly on the free surfaces of solids, without or along with the intermediate intervention of dissolved portions. It is obvious that such reactions will proceed more rapidly in proportion to the extent of the reactive surface. If it is remembered also that the disappearance or formation of colored solids, even in small amounts, is much more visible on the white surface of porous paper than in an aqueous suspension, it is easy to see that spot reactions involving solids which are highly dispersed in the capillaries of paper will lead to phenomena that will not be observed when the same reaction is carried out with compact particles in a test tube, or detectable there only by much more involved methods. The following experiments demonstrate this.

1. *The Reaction of Mercuric Sulfide with Alkali Iodide and Free Iodine*

Mercuric sulfide dissolves in potassium polyiodide (a solution of iodine in potassium iodide) with deposition of sulfur. This reaction is the basis of a volumetric (iodometric) method of determining mercuric sulfide. It can be represented:

$$HgS + 2KI + I_2 = K_2HgI_4 + S \qquad (1)$$

It has been commonly assumed that this reaction does not occur directly, but that (1) is merely the sum of (2) and (3), which take place in succession:

$$HgS + 4KI = K_2HgI_4 + K_2S \qquad (2)$$
$$K_2S + I_2 = 2KI + S \qquad (3)$$
$$HgS + 2KI + I_2 = K_2HgI_4 + S \qquad (1)$$

A spot reaction on paper impregnated with mercuric sulfide easily demonstrates that this assumption is incorrect. Contrary to expectation, iodine reacts *directly* on mercuric sulfide:

$$HgS + I_2 = HgI_2 + S \qquad (4)$$

and only then does the mercuric iodide dissolve in potassium iodide:

$$HgI_2 + 2KI = K_2HgI_4 \qquad (5)$$

Consequently, the reactions occur in the order (4) and (5).

Experiment. Filter paper is soaked in 1 per cent solution (alcohol) of mercuric chloride, dried, and then bathed in dilute ammonium sulfide solution. The gray-black paper, which is thus impregnated with mercuric sulfide, is washed with water and then finally dried. A drop of 0.2 *N* iodine solution in potassium iodide is placed on this mercuric sulfide paper and next to it a drop of 0.5 per cent solution of iodine in carbon disulfide. The specimen is dried in a current of warm air. After the paper is put in water, the area treated with iodine-potassium iodide solution becomes perfectly

white. This result corresponds to (1) which is the net equation of the reaction. The area spotted with the solution of iodine in carbon disulfide turns yellow-red, because of the formation of mercuric iodide. A white fleck is produced here also, but only after bathing the paper in a solution of potassium iodide. Consequently, the two spot reactions demonstrate the direct reaction of mercuric sulfide with iodine, and also the correct sequence of the reactions when polyiodide acts on mercuric sulfide; namely, the primary formation of HgI_2 followed by solution in potassium iodide to form K_2HgI_4.

2. *Transformation of Metal Sulfides to Insoluble Metal Iodides*

The conversion of mercuric sulfide into mercuric iodide, described in the foregoing experiment, is not an exception depending, for example, on the solubility of mercuric iodide in potassium iodide to produce complex K_2HgI_4. On the contrary, it is possible by means of spot reactions to demonstrate a direct conversion of other heavy metal sulfides into iodides. Dry filter paper impregnated with PbS, Ag_2S, Bi_2S_3, CuS, or Tl_2S, is spotted with one or two drops of a solution of iodine in carbon bisulfide or ether. After the solvent has evaporated and the specimen has been exposed to a blast of heated air, the formation of iodide will be evidenced by the change in color on the spots where the iodine was applied to the black paper. The following demonstration of the conversion of stannic sulfide into the iodide is quite simple.

Experiment. Stannic sulfide is prepared in a test tube by passing hydrogen sulfide into an acidified solution of stannic chloride. The precipitate is washed several times with water by decantation. The product is dissolved in the test tube by drop-wise addition of colorless ammonium sulfide and produces a solution of ammonium thiostannate. A drop of this solution is placed on filter paper and dried for about 2 minutes in a current of heated air. The sulfo salt is decomposed:

$$(NH_4)_2SnS_3 = SnS_2 + 2NH_3 + H_2S$$

A fleck of bright yellow stannic sulfide is left on the paper. If now a solution of iodine in carbon bisulfide or ether is applied, and the iodine driven off by warming, a red-brown fleck of stannic iodide remains. This procedure reveals quantities of stannic sulfide so small that they are not visible on the paper of themselves. It is recommended that similar trials be made with progressive dilutions of the ammonium thiostannate solution.

3. *Reactions of Finely Divided Manganese Dioxide*

Manganese dioxide is easily dissolved by concentrated hydrochloric acid, especially on warming:

$$MnO_2 + 4HCl = MnCl_2 + Cl_2 + 2H_2O$$

The course of the solution can be followed easily by the disappearance of the solid and also by the evolution of chlorine (bluing of starch-iodide paper). Dilute hydrochloric acid at room temperature reacts with manganese dioxide very slowly. This is also true for dilute solutions of other reducing agents, such as oxalic acid, sulfurous acid, hydrogen peroxide, and the like. Consequently, if precipitated manganese dioxide is shaken in a test tube with a mixture of dilute oxalic acid and dilute sulfuric acid, the consumption of manganese dioxide by the reaction with oxalic acid:

$$MnO_2 + H_2C_2O_4 + H_2SO_4 = MnSO_4 + 2CO_2 + 2H_2O,$$

cannot be seen, but can only be established by a quantitative determination. The findings are quite different, however, when manganese dioxide is finely dispersed in the capillaries of paper. If a drop of a dilute solution of a reducing agent is placed upon dry paper containing only a little manganese dioxide, the finely divided oxide reacts almost immediately. The reduction of the manganese dioxide on the spot where the reducing agent was applied results in a white fleck with a light brown or yellow border.

The presence of reducing agents can be plainly established by utilizing manganese dioxide paper. The reactivity of manganese dioxide when highly dispersed in the capillaries of paper is so great than even one drop of dilute sulfurous acid, indeed even moderately concentrated acetic acid, produces white flecks. Consequently, reactions can be seen under these circumstances which would be entirely invisible if carried out in test tubes.

Experiment. Papers with different manganese dioxide content can be prepared by bathing strips of quantitative filter paper in solutions of permanganate of different concentrations (from 1 per cent down), and also containing a little sodium hydroxide. The permanganate, depending on its concentration and the length of exposure, oxidizes the cellulose to a greater or less degree. Manganese dioxide is deposited in the capillaries of the paper and cannot be washed out. Papers from deep brown to barely visible yellow can be produced; after they have been washed thoroughly (bathed in running water) and dried, they will keep indefinitely. Of course, slightly impregnated papers should be used for the detection of minute quantities (up to 1γ) of reducing compounds. The reaction period in this case should be several minutes, so that the reduction of the manganese dioxide will be seen easily. If the paper is so slightly impregnated that it appears almost white, any action on the oxide can be made visible if the paper is spotted, dried, and then placed in an alcohol solution of benzidine. The latter is a sensitive reagent for traces of manganese dioxide, which colors it blue (see page 141). Consequently, on treatment with this reagent, the whole strip of paper turns intensely blue, with the exception of the fleck, which remains almost white.

4. *Reaction of Mercuric Oxide with Hydroxylamine Hydrochloride*

If an excess of mercuric oxide is added to a solution of hydroxylamine hydrochloride, and gently warmed, mercury is precipitated:

$$2\,NH_2OH\cdot HCl + 2HgO = Hg + HgCl_2 + N_2 + 4H_2O$$

This net equation represents a reaction that actually occurs in two stages. Hydroxylamine is liberated:

$$2\,NH_2OH\cdot HCl + HgO = HgCl_2 + H_2O + 2NH_2OH, \qquad (1)$$

and it then reacts with mercuric oxide, producing finely divided mercury:

$$HgO + 2NH_2OH = Hg + N_2 + 3H_2O \qquad (2)$$

The two partial reactions (1) and (2) can be easily segregated by the following spot reaction, and their separate courses made visible.

Experiment. Filter paper is impregnated with 0.2 *N* (alcohol) solution of mercuric chloride, dried, and then bathed, for a short time, in 1 *N* sodium hydroxide. After thoroughly washing with water, the paper is dried in a blast of heated air. The color will be a uniform yellow, due to the deposition of mercuric oxide. Mercuric oxide paper is stable only if it is stored in the dark (see page 94). A drop of hydroxylamine hydrochloride solution is placed on the paper and the capillary picture will go through the following stages: Proceeding from the point of application, a white circle is formed first [zone of partial reaction (1)] and adjoining this, a black ring, due to the deposition of mercury [zone of partial reaction (2)], the whole surrounded by unaltered yellow mercuric oxide. The formation of mercuric chloride in the white central circle can be proved by fuming with hydrogen sulfide, or by spotting with dilute potassium iodide solution; black mercuric sulfide, or red mercuric iodide, is formed.

The experiment can be made with hydrazine sulfate solution in place of hydroxylamine hydrochloride; analogous partial reactions occur.

5. *The Action of Iodine on Free Sulfur*

It is common practice to determine acid-soluble sulfides titrimetrically by adding the sample to an excess of acidified solution of known iodine content. The reaction:

$$H_2S + I_2 = 2HI + S \qquad (1)$$

occurs, and the unused iodine is then back-titrated with standard sodium thiosulfate solution. This titration involves a slight error because excess iodine can react with the finely divided sulfur produced in (1) and thus may lead to an oxidation to sulfate:

$$S^0 + 3I_2 + 4H_2O = 8H^+ + 6I^- + SO_4^{--} \qquad (2)$$

The extent of this side reaction (2), which entails a consumption of iodine in excess of that demanded by (1), is extremely small. In practice it can be neglected if the determination is carried out competently, that is, if the excess of iodine is immediately back-titrated. However, the demonstration of the oxidation of sulfur to sulfate makes an interesting spot reaction because, in a simple way, it illustrates an effect which is a matter of everyday experience in the case of the other halogens, bromine and chlorine.

Experiment. A drop of a solution (1 per cent) of sulfur, of the highest grade, in carbon disulfide is placed on neutral litmus paper. After the solvent has evaporated the spot is treated with one drop of 0.5 *N* iodine solution (in potassium iodide). For comparison, a drop of carbon disulfide is placed on the same strip of indicator paper, and after evaporation, a drop of the iodine solution is applied on the same place. The litmus paper is then placed in water; the iodine dissolves, and a red fleck will be seen, but only at the place where evaporation of the carbon disulfide deposited free sulfur, which then came in contact with the iodine. This demonstrates that even brief action of iodine (and water) results in an oxidation of sulfur to sulfate (sulfuric acid) as given in (2).

6. *The Action of Mercuric Oxide and Chloride on Filter Paper in Ultraviolet Light*

Mercuric oxide and mercuric chloride can be reduced by certain organic and inorganic compounds; free mercury or a mercurous salt are produced, depending on the conditions of the experiment. Cellulose of filter paper can reduce these mercury compounds partially to metallic mercury; irradiation, particularly with ultraviolet light (quartz lamp), catalytically accelerates this reduction. The rapid discernment of the reaction of cellulose with mercuric oxide or chloride requires that the mercury compounds be highly dispersed in the capillaries of the paper. This is accomplished by impregnating filter paper with mercuric oxide or chloride.

Experiments

1. Strips of filter paper are placed in 0.2 *N* alcohol solution of mercuric chloride. The alcohol is allowed to evaporate and mercuric oxide is deposited in the capillaries of the paper by placing the strips in 1 *N* sodium hydroxide. The yellow paper is thoroughly washed with water and dried in a blast of heated air. It can be kept for a long time if stored in the dark.

A strip of mercuric oxide paper is placed on a glass plate and a portion of the paper is covered with a coin. The paper is then exposed to the light of a quartz lamp. After about three minutes, the exposed areas of the paper became uniformly gray, whereas the protected area is unaltered. The under side of the paper also remains practically unchanged, indicating

that the layer of finely divided mercury produced by the reduction protects the underlying mercuric oxide from the action of the ultraviolet rays.

If the paper is left under the quartz lamp for an hour, or if it is exposed to the prolonged action of direct sunlight, the reaction proceeds so far that the irradiated paper is blackened uniformly by the deposition of finely divided mercury.

2. The action of mercuric chloride is made visible by placing a drop of 0.1 *N* mercuric chloride solution on filter paper and then placing the paper under the quartz lamp. Within several minutes the exposed side of the fleck becomes black-brown. If a drop of water is applied at the side of the brown fleck, that part of the paper which is moistened by the spreading water becomes colorless immediately. The reaction

$$Hg + HgCl_2 = Hg_2Cl_2$$

occurs in this zone. The finely divided free mercury, produced by the action on cellulose (when irradiated), reacts with the unchanged mercuric chloride left in the paper and immediately forms insoluble white mercurous chloride. The formation of calomel can be proven by holding the paper over ammonia water or by spotting with this reagent. The decolorized areas which contain mercurous chloride turn deep black:

$$Hg_2Cl_2 + 2NH_3 = Hg + HgClNH_2 + NH_4Cl,$$

a reaction which is characteristic of calomel.

7. *The Action of Alkali Polysulfide and Free Sulfur on Thallous Sulfide*

This section describes a number of original experiments carried out as spot reactions. The order is that in which they were actually performed during an investigation in this laboratory. The resulting observations and their useful application have led to a new sensitive test for free sulfur, and have also revealed the conditions under which a hitherto unknown sulfur compound of thallium is formed.

Experiments

Filter paper is impregnated with 0.1 *N* thallous carbonate solution and while still moist is held over a beaker containing ammonium sulfide. Black thallous sulfide is deposited on the paper and in its capillaries. The paper is washed briefly, dried in heated air, and can then be kept for several days in a tightly fitting container. Long standing, especially in moist air, leads to oxidation of the thallium sulfide to sulfate.

A drop of dilute ammonium polysulfide solution is placed on a strip of thallium sulfide paper, dried, and then bathed in dilute nitric acid. A brown-red residue appears on the spotted area, while the rest of the paper

turns white because of the ready solubility of thallous sulfide in acids. (The ammonium polysulfide can be replaced by alkali polysulfide, and the dilute nitric acid by other dilute mineral acids.) If the experiment is made with colorless ammonium sulfide no residue is left, but the entire paper loses its color completely.

These two experiments lead to the conclusion that the polysulfide sulfur is solely responsible for the difference. The spotting is repeated with a drop of strong polysulfide solution which contains much sulfur. Brown acid-resistant flecks are produced on the black paper if it is allowed to lie in the air, or the effect is observed more quickly if a blast of heated air is directed against the paper.

This finding makes it appear probable that when ammonium polysulfide evaporates, sulfur is deposited on the thallium sulfide and protects it from attack by the acid (pp. 86 and 229). The spot experiment is therefore repeated several times, but after the paper has been dried it is bathed in carbon disulfide or colorless ammonium sulfide to dissolve away the free sulfur. It will be found that, in spite of the action of the excellent solvent for sulfur, the flecks themselves persist and retain their resistance to acid. A noteworthy difference in behavior of the flecks that have only been dried, and of those which received additional treatment with carbon disulfide, was observed. The former produced a red-brown fleck on a white background when bathed in acid; the latter (that is after digestion with carbon disulfide) formed a brown fleck on a light brown background. These observations indicate that sulfur dissolved in carbon disulfide exerts an effect, and lead to the spotting of thallium sulfide paper with a drop of a carbon disulfide solution of sulfur. Immediately after the solvent evaporated, the brown acid-stable flecks, previously observed, appear. When the flecks produced by spotting with a solution of sulfur in carbon disulfide were bathed in this solvent and then treated with acid, the brown flecks on a light brown background remain again.

These experiments prove that dissolved sulfur reacts very rapidly with finely divided thallous sulfide, and that excess sulfur dissolved from the fleck by carbon disulfide reacts immediately with the unchanged thallous sulfide that is still present around the fleck. Consequently, it is possible, as shown by the spot experiments with thallous sulfide finely divided in paper, for black thallous sulfide to take up free sulfur from carbon disulfide or polysulfide solutions, and form a red-brown product which is acid-stable in contrast to thallous sulfide. The great tendency to combine with sulfur points to the formation of stable thallium polysulfide $Tl_2S \cdot S_x$ or to a new modification of thallic sulfide. A synthesis of the compound by treatment of dry Tl_2S with a solution of sulfur in carbon disulfide or with polysulfide solutions seems to have promise.

If thallous sulfide is precipitated in the ordinary fashion, dried and then allowed to stand, with occasional shaking, in contact with a solution of sulfur in carbon disulfide, the color changes toward brown only after 24 hours. The high dispersion of thallous sulfide in paper has therefore made it possible to discover a reaction which hitherto could not be observed because it proceeds so slowly.

The effect of the combining of sulfur with thallous sulfide can also be seen if thallous sulfide paper is spotted with a very dilute solution of sulfur in carbon disulfide and then treated with acid. A brown fleck will be left on the decolorized paper. Small quantities (1γ) of sulfur in carbon disulfide can be detected in this way.

Bright brown or dark brown flecks are left if thallous sulfide paper is spotted with a solution of selenium in carbon disulfide or colorless ammonium sulfide, dried, and then bathed in dilute acid. This finding proves definitely the transformation of the finely divided thallous sulfide into a compound containing selenium. The same effect is obtained in greater measure when the spotting is done with a solution of selenium in colorless ammonium sulfide.

F. Accumulation of Materials by Extraction and Flotation

Materials dissolved in water, or solids suspended in this medium, can be quickly and extensively removed from the water phase by an immiscible organic solvent (ether, amyl alcohol, carbon disulfide, etc.), if the material is quite soluble in the organic liquid. A transient intimate mixing is essential, so that close contact with the organic solvent is provided by the development of an extensive mutual surface. If the system is then allowed to stand until the liquids separate, the water-organic liquid interface will again assume its minimum area and the material which was originally distributed in the water will then be dissolved in the organic solvent. The extraction is complete if the materials are insoluble in water, but a complete extraction can never be attained with water-soluble compounds. A partition between both solvents is the best that can be accomplished. The distribution depends on the relative solubility of the particular compound in water and in the organic liquid. Extraction by an organic solvent of materials dissolved or suspended in water is called "shaking out." This procedure is particularly adaptable to the detection of minute quantities of colored materials that are soluble in organic liquids; the material originally distributed over a large volume can be localized by extraction into a much smaller volume and thus made more visible. Colorless organic liquids with high solvent power should be chosen for shaking out; their densities should be somewhat different from that of water, so that the layers will form quickly. As a rule, the layers separate more rapidly after acid or

neutral solutions have been extracted than when alkaline solutions are shaken out. Water-alcohol solutions often form stable emulsions when they are shaken out with organic solvents. In such cases, it is well to dilute with water either before or after the extraction; the layers then usually separate more rapidly.

Freshly precipitated, and consequently finely divided, materials that are not soluble in water, nor in immiscible organic solvents, frequently can be collected in the interface of the two liquids if the suspension is shaken vigorously, and the layers then allowed to separate. A fine film is formed on the surface of the water which is in contact with the organic liquid, and thus small quantities of colored materials, which are difficult to see in a large volume, can be discerned clearly. This type of localization of material is similar to the "flotation" that is so widely used in chemical industry. It is a procedure for separating the components of a mixture of pulverized materials by floating them in water. The addition of small quantities of certain materials (flotation agents) alters both the wettability of the particles and the surface tension of the liquids, and therefore changes the buoyancy also. Flotation of freshly precipitated materials can sometimes be used for analytical purposes; this is accomplished by extraction with organic solvents, since not only water but also organic liquids, such as ether, can be adsorbed on finely divided solids. If the adsorption of two liquids is sufficient to increase their miscibility on the surface, the solid will collect at the water-organic liquid interface. This accumulation, therefore, is always an indication of the common adsorption of two immiscible liquids on the surface of a solid phase. If water alone is adsorbed, then the solid remains in the water after the shaking out. On the other hand, if the adsorption of an organic liquid is greater than that of the water, then the solid phase goes into the organic layer on shaking out. A localization of material by flotation is especially advantageous if colored reagents, which interfere with the discernment of small quantities of colored precipitates, are taken up by the organic liquid. In such cases, shaking out accomplishes both extraction and flotation. Ether is a good flotation agent. Sometimes organic liquids whose density is greater than that of water, such as carbon disulfide or carbon tetrachloride, can be used with excellent results. As a rule, the presence of neutral salts and an acid reaction of the test solution favor a flotation of the precipitate.

Finally, a third method of localizing material consists in shaking dilute salt solutions with small quantities of solid materials which are capable of adsorbing certain components of the solution and thus retaining them. Sometimes the retention is due to chemical reaction. Materials which can exert such effects are called "trace catchers." If a dilute solution is shaken with a suitable trace catcher, and the liquid poured off, a chemical change

can be detected directly by the change in color of the catcher, or the latter can be spotted with a suitable sensitive reagent. A positive result of the characteristic reaction will thus establish that an adsorption has taken place.

Experiments

1. A standard ferric sulfate solution (1 ml. = 0.001 g. Fe) is treated with 1 ml. of concentrated hydrochloric acid and enough water to produce a 1:20 solution. Of this, 2 ml. are treated in a narrow test tube with 1 ml. of 10 per cent potassium thiocyanate solution. A distinct red color, due to $Fe(CNS)_3$ or $Fe(CNS)_6^{---}$ or $Fe(CNS)_2^{+}$, is obtained. A portion of the red solution is diluted with hydrochloric acid (1:10) until a pink can be barely seen. This solution is shaken thoroughly with 1 ml. of ether. After the layers have separated, the upper layer will be distinctly pink, because the ether has extracted the greater part of the ferric thiocyanate; the water layer will be almost colorless. The red or pink can be discharged by adding, from the tip of a knife blade, a trace of potassium fluoride, which forms the colorless complex FeF_6^{---} ion.

2. A 1:20 diluted solution is prepared by adding 1 ml. of concentrated hydrochloric acid and sufficient water to standard cobalt nitrate solution (1 ml. = 0.001 g. Co). Two ml. of the dilute solution are treated in a narrow test tube with about 1 g. of potassium thiocyanate. The solution turns blue-green. Several drops of acetone and 1 ml. of ether are added and the mixture thoroughly shaken. When the layers separate, the ether layer will be intensely blue. The color is due to the formation of the complex salt $K_2Co(CNS)_4$, which is soluble in ether-acetone.

Further dilutions of the diluted cobalt nitrate solution are prepared and tested in the same way with potassium thiocyanate and ether + amyl alcohol. It will be found that cobalt solutions which no longer change color on the addition of potassium thiocyanate will give a distinct blue upper layer.

The difference in the sensitivity of the thiocyanate test for cobalt, with and without extraction, is far greater than the analogous difference in the detection of ferric thiocyanate. This is in accord with the fact that even dilute solutions contain ferric thiocyanate as such, whereas, in dilute cobalt solutions, the formation of $K_2Co(CNS)_4$ is only partial, in conformity with the equilibrium:

$$Co^{++} + 4CNS^{-} \rightleftharpoons Co(CNS)_4^{--}.$$

If, however, this equilibrium solution is shaken with ether + acetone, the double compound is withdrawn from the water phase, and the complex cobalt thiocyanate, which dissolves in the organic solvent, with a blue

color, is replenished. The blue complex cobalt thiocyanate, in contrast to red ferric thiocyanate, is stable toward potassium fluoride. This fact makes it possible to detect traces of cobalt in the presence of much iron.

3. Fifteen ml. of distilled water are placed in a test tube provided with a glass stopper, and a like volume of tap water is placed in a similar test tube. Three ml. of 0.002 per cent solution of dithizone in carbon tetrachloride are added to each test tube and the mixtures shaken. The original green of the dithizone solution changes to violet-red in both cases, but the change is far more intense in the tap water than in the distilled water. Tap water, and even distilled water, contains traces of heavy metals that react with dithizone and form colored compounds which are soluble in carbon tetrachloride. Accordingly, the presence of traces of heavy metals in water can be detected by shaking the specimen with dithizone reagent solution. After the layers have separated, several ml. of the distilled water are transferred to another test tube and shaken again with 2 ml. of dithizone solution. This method of purifying water is repeated until the carbon tetrachloride layer no longer undergoes a color change. A lead wire is then hung for several minutes in the water from which the heavy metals have been removed. On shaking with dithizone, the water will turn red again. The traces of lead salt formed on contact of the water with the wire are sufficient to give a test with dithizone.

4. Two milliliters of 0.001 per cent solutions of sodium chloride and potassium iodide, in separate test tubes, are treated with 2 drops of 0.1 *N* silver nitrate solution and 1 drop of dilute nitric acid. An opalescence appears, due to the formation of silver chloride and silver iodide. A visible deposit is not obtained for several hours because the silver halides are so finely divided. The experiments are repeated, but the opalescent liquids are shaken with 1 ml. of ether immediately after the silver nitrate has been added. When the layers have separated, a film of silver chloride or silver iodide will be seen at the water-ether interface. The improvement in the visibility of the silver halide precipitation by shaking with ether is demonstrated easily by trials with progressively diluted solutions of sodium chloride and potassium iodide.

5. Ammoniacal copper sulfate solution is diluted until 5 ml. treated with colorless ammonium sulfide produces neither a precipitate nor a turbidity. Five milliliters of this diluted copper solution are shaken vigorously in a stoppered hard glass test tube with a grain of silica gel for 5 minutes. The liquid is poured off and the silica gel transferred to a depression of a spot plate. A drop of ammonium sulfide solution is added. A visible production of copper sulfide follows; the silica gel has adsorbed the copper-ammine salt and thus localized the copper on a small surface.

Chapter IV

INORGANIC ANALYSIS

A. Spot Tests with Inorganic Reagents

(Normal Salts and Complex Compounds)

A comparatively minor rôle is played by inorganic reagents in inorganic spot analysis. Their reactions with the materials to be detected produce simple salts which are either slightly soluble or soluble and colored. The scarcity of inorganic binary salts useful as reaction products is definitely typical of spot analysis in direct contrast to classical qualitative macro- and micro-analysis, in which such salts are used in large measure because they frequently exhibit characteristic crystalline form. The infrequent use of inorganic reagents stems from the fact that spot tests are limited to reactions which are sensitive and as selective as possible, and whose positive results can be established without the aid of optical instruments which must be called on, for instance, to determine the crystalline form of the reaction product. These requirements are met far better by tests accomplished by means of organic reagents, catalyzed reactions, masking agents, and so forth, than they are by the normal formation of inorganic salts. Inorganic compounds are of importance in spot tests if they participate in reactions involving complex salts. Included in this class of reagents are those compounds which react with the materials being identified by forming complex salts whose color or slight solubility can be utilized for analytical purposes. Further examples are those inorganic reagents which are themselves complex compounds, and as such behave quite differently than their constituents. Lastly, the color intensification undergone by some inorganic components when they are incorporated in certain adsorption systems can be made to serve the needs of spot tests.

Inorganic reagents are essential aids in the preparation of solutions of the samples, in the segregation (precipitation, masking) of certain groups or co-solutes from mixtures, in providing a suitable reaction environment (pH adjustment), or in the preliminary attainment of the appropriate stages of oxidation of materials whose identification reactions are shown only at certain valences.

1. Detection of Antimony (III) and Tin (II)

Chemical Basis: Reduction of soluble and slightly soluble phosphomolybdates. Normal molybdates are such weak oxidizing agents that they do not affect antimony (III) salts. However, molybdenum attains an enhanced reactivity in phosphomolybdic acid, $H_3PO_4 \cdot 12MoO_3 \cdot 2H_2O$, and

its salts because of the complex binding of MoO_3 molecules. In this state it can be reduced quickly by certain reducing agents which either do not affect normal molybdate or do so only slowly and incompletely. Blue soluble molybdic oxides, "molybdenum blue," result. Molybdenum blue is a mixture of MoO_3 and Mo_2O_5; consequently, it always represents no more than a partial reduction of Mo (VI). Antimony (III) salts can cause this reduction. It is noteworthy that they react only with free phosphomolybdic acid or its soluble salts, but not with slightly soluble salts such as potassium or ammonium phosphomolybdate. In contrast, tin (II) salts reduce both soluble and slightly soluble phosphomolybdates. Consequently, filter paper impregnated with yellow acid-insoluble ammonium phosphomolybdate can be used for spot detecting tin in the presence of antimony.

Procedure: Immediately before the experiment filter paper is impregnated with a saturated alcohol solution of phosphomolybdic acid and then dried. The paper will keep for only a few days, even when stored in the dark. A drop of the acid test solution is placed on the yellow reagent paper, which is then exposed to steam for several minutes. If antimony is present a blue spot develops; its intensity is an index of the quantity of antimony.

Permanent ammonium phosphomolybdate paper suitable for the detection of tin (II) is prepared by immersing paper impregnated with phosphomolybdic acid in an aqueous solution of ammonium nitrate acidified with nitric acid, washing with water, and drying. A drop of tin (II) solution immediately produces a blue spot on this yellow reagent paper.

Limit of Identification: 0.2 γ antimony.

0.03 γ tin.

Concentration Limit: 1:250,000 antimony.

1:600,000 tin.

Application in the presence of other materials: The test for antimony (III) and tin (II) is unequivocal if other reducing agents are absent. If antimony is to be detected in the presence of other cations, including tin, it is well to boil the solid sample, or a solution prepared from it, with a solution of alkali sulfide. This converts arsenic, antimony, and tin into soluble alkali thio compounds, such as Na_3SbS_4 or Na_3SbS_3. The residual sulfides are removed by filtration or centrifugation. A few drops of the filtrate or centrifugate are boiled with a little concentrated sulfuric acid until a clear solution is obtained. Antimony (III) sulfate and tin (IV) sulfate result. The test for antimony can be made directly on this solution; if necessary while it is still warm.

The detection of tin is made best in this solution of antimony (III) and tin (IV) sulfate. A few drops are heated in a porcelain crucible until

sulfuric acid vapors are evolved. After diluting with water, the tin is reduced by adding a few granules of zinc; metallic antimony is deposited as a black powder. A drop of the solution thus prepared is placed on phosphomolybdate paper. The precipitated antimony does not interfere with the detection of tin.

2. Detection of Copper

Chemical Basis: Formation of mixed crystals of $CuHg(CNS)_4$ *and* $ZnHg(CNS)_4$. Solutions of copper or zinc salts treated with solutions of complex alkali mercuric thiocyanates such as $(NH_4)_2Hg(CNS)_4$, produce crystalline precipitates of $CuHg(CNS)_4$ (green) or $ZnHg(CNS)_4$ (white). If these complex thiocyanates are precipitated separately and then mixed a bright green product results. If, however, solutions containing both zinc and copper are treated with an alkali mercuric thiocyanate and the complex thiocyanates coprecipitated, the result is quite different. The crystalline precipitate is deep violet. The abnormal color is due to the union of the complex thiocyanates, which form violet mixed crystals. Even very small quantities of the copper salt can produce these mixed crystals and so cause a characteristic alteration of the white $ZnHg(CNS)_4$.

Procedure: A drop of the acidified test solution is placed on the spot plate and treated with one drop each of 1% zinc acetate and ammonium mercuric thiocyanate solution. If copper is present a violet precipitate of zinc mercuric thiocyanate will be obtained on mixing with a fine glass rod. After the precipitate has settled on the white background the coloration can be detected easily, even though only minute quantities of copper are present. If the test appears uncertain, it is well to repeat the attempt with smaller quantities of zinc and compare the result with a blank test. The ammonium mercuric thiocyanate solution contains 8 g. $HgCl_2$ and 9 g. NH_4CNS in 100 ml. water.

Limit of Identification: 0.1 γ copper.

Concentration Limit: 1:500,000.

Application in the presence of other materials: Iron (III) salts form a red solution with thiocyanate, and they also color $ZnHg(CNS)_4$. The precipitate cannot be decolorized even by long continued washing with water. This interference can be avoided if an alkali fluoride is added to the acid solution before the precipitation is made. The complex FeF_6^{---} ion is formed; it does not react with thiocyanate ion. Cobalt and nickel salts also interfere because they form green $CoHg(CNS)_4$ and blue $NiHg(CNS)_4$, which mask the copper reaction. If copper is to be detected in mixtures containing cobalt and nickel also, it is best to treat the acidified solution with hydrogen sulfide and ignite the mixture of sulfides. The residue is dissolved in dilute nitric acid and the test then carried out.

3. Detection of Magnesium

Chemical Basis: Formation of an adsorption compound of magnesium hydroxide and iodine. Freshly precipitated magnesium hydroxide is turned deep brown by iodine + potassium iodide solution. The color is discharged on digestion with solvents which dissolve iodine (potassium iodide, alcohol, other organic solvents), or on the addition of sulfite or thiosulfate. The system therefore obviously presents an adsorption of free iodine on $Mg(OH)_2$, which can also adsorb many other materials, particular dyes, with intensification or modification of the color (cf. p. 7). This same adsorption compound likewise results if magnesium hydroxide is precipitated in the presence of free iodine. The most favorable conditions for this are attained in dilute magnesium solutions when iodine-iodide solutions, decolorized with caustic alkali just before the precipitation, are used. This hypoiodite solution presents the equilibrium:

$$2\,I + 2\,OH^- \rightleftarrows I^- + IO^- + H_2O.$$

Hydroxyl ions are removed from the solution by the precipitation of $Mg(OH)_2$ and the free iodine required for the adsorption is thus made available. The use of freshly prepared hypoiodite is further necessitated because on long standing the reaction:

$$3\,IO^- \rightleftarrows IO_3^- + 2\,I^-$$

occurs, and this consumes the hypoiodite ions furnishing the iodine.

Procedure: A drop of the neutral or acidified test solution is treated on a spot plate successively with one microdrop of 1 *N* potassium hydroxide and 1 *N* iodine solution. The three drops are then mixed with a thin glass rod. The solution should have a distinct brown color (free iodine) and consequently more iodine solution (1 *N* in 20 per cent KI) should be added if necessary. After about one minute, 1 *N* alkali is added from a micropipette until the solution has become lemon yellow. If magnesium is present brown flakes of the iodine adsorption compound can be seen in the yellow solution. If very small quantities of magnesium are suspected it is best to run a blank test.

Identification Limit: 0.3 γ magnesium.

Concentration Limit: 1:165,000.

Application in the presence of other materials: The hypoiodite reaction is quite selective for magnesium. It is impaired by the presence of metal ions which form colored hydroxides, and by considerable quantities of reducing agents and ammonium salts. In the latter cases, it is best to evaporate one or two drops of the sample in a porcelain crucible, ignite, take up the ignition residue in dilute acid, and make the test on this solution.

4. Detection of Mercury

Chemical Basis: Formation of red Cu_2HgI_4. If an equimolar mixture of white cuprous iodide and red-yellow mercuric iodide is suspended in water and heated the color deepens because the two iodides unite to form the dark red slightly soluble iodide Cu_2HgI_4. This compound is also formed when solutions of mercuric salts react with cuprous iodide suspended in them:

$$Hg^{++} + 2\ Cu_2I_2 \rightarrow Cu_2HgI_4 + 2\ Cu^{+}.$$

This reaction can be utilized as the basis of a spot test for mercury. It is better to use reagent paper impregnated with white slightly soluble Cu_2I_2, on which a color change due to the formation of the complex iodide is easily noticed.

Procedure: A drop of the neutral or slightly acid test solution is placed on filter paper impregnated with Cu_2I_2. A pink to red coloration develops according to the quantity of mercury present. Cuprous iodide paper is prepared by soaking filter paper in $CuSO_4$ solution and then dipping it into a warm solution of KI containing H_2SO_3. When washed and dried, the paper is stable.

Identification Limit: 0.03 γ mercury.

Concentration Limit: 1:1,600,000.

Application in the presence of other materials: Palladium, which forms black PdI_2, also reacts under the foregoing conditions. Considerable quantities of bismuth salts (more than 500 mg. per liter) form brown BiI_3. The sensitivity of the test is lower if the acidity exceeds 0.1 *N*.

5. Detection of Bismuth

Chemical Basis: Formation of slightly soluble red $BiCr(CNS)_6$. If bismuth salts, in mineral acid solution, are treated with an aqueous or alcoholic solution of complex potassium chromic thiocyanate and warmed, a crystalline brick red precipitate of bismuth chromic thiocyanate is formed:

$$Bi^{+++} + Cr(CNS)_6^{---} \rightarrow BiCr(CNS)_6.$$

Procedure: A microdrop (0.012 ml.) of the test solution is placed on filter paper and dried over a heated asbestos plate or in a current of warm air. A drop of 3 per cent alcoholic solution of $K_3Cr(CNS)_6$ is then applied, the drying repeated, and a drop of sulfuric acid (1:2) added. A brick red spot or ring is produced, depending on the quantity of bismuth present.

Identification Limit: 0.4 γ bismuth.

Concentration Limit: 1:31,000.

Application in the presence of other materials: This test for bismuth is quite selective as, under these conditions, analogous complex thiocyanates

are formed only by mercury, silver, thallium, and lead. Their products, however, are not as highly colored, being pink or yellow.

6. Detection of Hydrazine and Hydroxylamine

Chemical Basis: Reduction of phosphomolybdic acid. Hydrazine and hydroxylamine are frequently used as reducing agents. The actions may be represented:

$$2\,NH_2OH + O \rightarrow N_2 + 3\,H_2O$$
$$N_2H_4 + 2O \rightarrow N_2 + 2\,H_2O.$$

Hydrazine is the stronger reducing agent of the two; this fact is exhibited under suitable conditions in its behavior toward phosphomolybdic acid. Both hydroxylamine and hydrazine will reduce phosphomolybdic acid to "molybdenum blue" in acetic acid solution (cf. p. 101). When heated, the sulfates of both bases react in the same manner upon phosphomolybdic acid, but at different rates. In the cold, however, only hydrazine sulfate effects this reduction, while hydroxylamine sulfate does not react.

Procedure: A drop of weakly acidified hydrazine sulfate solution is placed in a depression of a spot plate and treated with a drop of 5 per cent aqueous solution of phosphomolybdic acid. On standing, the color changes, depending on the quantity of hydrazine, through green yellow and green to blue. A parallel test with a drop of water is recommended if the quantity of hydrazine is small.

Identification Limit: 1 γ hydrazine.

Concentration Limit: 1:50,000.

Application in the presence of other materials: Since this test is based solely on the difference in the redox potentials of hydrazine and hydroxylamine, other stronger reducing agents, such as iron (II), antimony (III), tin (II), or sulfite, must be absent. It should be noted also that potassium and ammonium salts react with phosphomolybdic acid to produce yellow crystalline precipitates, which are not affected by hydrazine. Consequently, the reduction of phosphomolybdic acid by hydrazine is especially recommended as a simple means of differentiating it from hydroxylamine.

7. Detection of Hydrazoic Acid

Chemical Basis: Formation of silver or ferric azide. Aqueous solutions of hydrazoic acid or its alkali salts react toward silver nitrate in much the same manner as halide solutions. Silver azide, AgN_3, precipitates. This does not dissolve in acid, but is soluble in ammonia, from which it can be re-precipitated by dilute nitric acid. Azide ion resembles thiocyanate ion particularly, because it also forms a soluble red ferric salt, FeN_9, which, like ferric thiocyanate, is quite soluble in ether.

The great volatility of hydrazoic acid, which can be expelled from its aqueous solutions even at 37°C., makes it possible to distinguish between azides and halides. Because of this volatility it can be isolated from all halogen hydracids and tested separately.

Procedure: A drop of the test solution is brought into the lower part of the apparatus (Fig. 25) described on p. 51, and treated with one or two drops of dilute hydrochloric acid. A drop of dilute silver nitrate or ferric chloride solution is placed on the glass knob of the stopper, the apparatus is closed, and warmed slightly on the heating block (p. 46). A turbidity, or the development of a red color, shows the presence of hydrazoic acid.

Identification Limit: 5 γ sodium azide.

Concentration Limit: 1:10,000.

Application in the presence of other materials: The test is specific for azide. Any interference due to sulfides, sulfites, or thiosulfates which are decomposed by acids and so give rise to Ag_2S or Fe (II) salt can be avoided if the test solution is subjected to a preliminary oxidation by adding several drops of 3 per cent hydrogen peroxide. The hydrogen peroxide must be neutral; if necessary it should be shaken with calcium carbonate before it is used.

8. Detection of Carbonic Acid

Chemical Basis: Decolorization of a sodium carbonate solution containing phenolphthalein. Soluble carbonates are alkaline to phenolphthalein in contrast to bicarbonate solutions, which are neutral toward this indicator. Consequently, a solution of Na_2CO_3 becomes red on the addition of phenolphthalein, whereas $NaHCO_3$ solutions remain colorless. Accordingly, a dilute carbonate solution colored with phenolphthalein is rapidly decolorized when carbon dioxide is passed through it:

$$Na_2CO_3 + CO_2 + H_2O \rightarrow 2\ NaHCO_3.$$

This fact forms the basis of a rather sensitive test for carbonates. The carbon dioxide liberated from them by acids is caught in a dilute solution of sodium carbonate colored red with phenolphthalein, and the discharge of the color constitutes a distinct test for this gas.

Procedure: The gas evolution apparatus (Fig. 25) described on p. 51, is charged with one or two drops of the test solution, or as much of the solid substance as covers the tip of a knife blade, and then three drops of 2 *N* sulfuric acid introduced. The apparatus is then closed with the stopper after a drop of sodium carbonate reddened with phenolphthalein has been placed on it. The color will be discharged immediately or after standing a short time, depending on the quantity of carbon dioxide released. It is best to carry out a blank test in a second apparatus to eliminate the possibility of false conclusions due to the carbon dioxide of the air. The

reagent is prepared by adding 2 ml. of 0.5 per cent alcoholic solution of phenolphthalein to 1 ml. of sodium carbonate solution and then adding 10 ml. of water.

Identification Limit: 4 γ carbon dioxide.

Concentration Limit: 1:12,500.

Application in the presence of other materials: The decolorizing reaction just described constitutes an unquestionable test for carbonates only in the absence of other materials which are decomposed by acids to evolve volatile acids. Consequently, if cyanides, azides, sulfides, sulfites, thiosulfates, and nitrites are present it is necessary to convert them into acid-resistant compounds by appropriate salt formation or by oxidation. The necessary reactions can be carried out in the evolution apparatus itself before liberating the carbon dioxide.

Cyanides and *azides* are converted into acid-resistant AgCN or AgN_3 by adding several drops of saturated silver nitrate solution. It is then possible to detect 5 γ carbon dioxide in the presence of 2500 times this quantity of cyanide or azide.

Sulfides, sulfites, and *thiosulfates* are rendered non-interfering by the addition of several drops of hydrogen peroxide, which oxidizes them quantitatively to sulfate. In this way, 5 γ carbon dioxide can be detected in the presence of 10,000 times this quantity of K_2S or Na_2SO_3 and of 20,000 times this amount of $Na_2S_2O_3$.

Fluorides can be converted into acid-resistant ZrF_6^{--} ions by adding concentrated zirconium chloride solution. It is possible then to detect 5 γ carbon dioxide in the presence of 1,000 times this quantity of K_2F_2.

Nitrites are rendered non-interfering by the addition of aniline hydrochloride, which consumes the interfering nitrous acid quantitatively through diazotization of the aniline. In this way, 5 γ carbon dioxide can be detected in the presence of 1,000 times this quantity of KNO_2.

Formates and *acetates* in considerable quantities interfere with the carbonate test by liberating the respective acids. The only feasible method of avoiding this complication is to precipitate calcium carbonate and test this separately. This may be done by placing the test solution in the bulb of the gas evolution apparatus and adding calcium chloride solution. Even minute quantities of the precipitate can be collected by centrifuging if the bulb, together with its liquid contents, is placed in a centrifuge tube.

9. Detection of Nitrate and Nitrite

Chemical Basis: Formation of nitroso-ferrous sulfate. Oxidation in acid solution by nitrates or nitrites always produces nitric oxide:

$$2\,HNO_3 \rightarrow H_2O + 3\,O + 2\,NO.$$
$$2\,HNO_2 \rightarrow H_2O + \;\;O + 2\,NO.$$

Nitric oxide combines with iron (II) to form the brown $FeNO^{++}$ ion, which is stable for some time at room temperature in concentrated acid solution. If, therefore, solid ferrous sulfate is used to reduce nitrate or nitrite in acid solution, the ferro-nitroso compound will form a brown ring around the unaltered ferrous sulfate. The reactions in the case of nitrates can be represented:

$$2\,HNO_3 + 6\,FeSO_4 + 3\,H_2SO_4 \rightarrow 3\,Fe_2(SO_4)_3 + 4\,H_2O + 2\,NO.$$
$$2\,FeSO_4 + 2\,NO \rightarrow 2\,[FeNO]SO_4.$$

Nitrites behave similarly.

Because of the ease with which nitrites are decomposed by acids, this "ring test" can be carried out even in acetic acid solution. Nitrates require the addition of concentrated sulfuric acid. Consequently, nitrites can be distinguished from nitrates by the formation of $FeNO^{++}$ ion in acetic acid solution, while nitrates can be identified in the presence of nitrites because the brown ring formed in acetic acid solution is intensified if concentrated sulfuric acid is added. It should be noted in this connection that even pure nitrites always contain some nitrate because of partial auto-oxidation.

Procedure: A crystal of ferrous sulfate, the size of a pin head, is treated in a depression of a spot plate with a drop of the test solution and then a drop of concentrated sulfuric acid is flowed in from the side. A brown ring around the ferrous sulfate crystal shows the presence of nitrate.

A drop of concentrated acetic acid is substituted for the sulfuric acid when testing for nitrite.

Identification Limit: 2.5 γ nitric acid.

Concentration Limit: 1:20,000.

Application in the presence of other materials: Iodides and bromides interfere because they give off iodine or bromine. It is necessary, then, to remove the halide ions by precipitating the corresponding silver salts by means of a saturated solution of silver sulfate or acetate. The sensitivity of the test is lowered by large quantities of chlorides because of the formation of nitrosyl chloride, NOCl. In addition, cyanides, ferrocyanides, thiocyanates, chromates, sulfites, thiosulfates, and iodates interfere because they react with ferrous sulfate and concentrated sulfuric acid and give rise to phenomena which make it more difficult to recognize the characteristic $FeNOSO_4$ ring. Molybdates or tungstates interfere because they are reduced to colored lower oxides by ferrous sulfate.

10. Detection of Hydrogen Peroxide and Peroxides

Chemical Basis: Conversion of lead sulfide to lead sulfate. Black lead sulfide is converted into the white sulfate, which is not soluble in water, by the action of hydrogen peroxide and peroxides:

$$PbS + 4\,H_2O_2 \rightarrow PbSO_4 + 4\,H_2O.$$

Precipitated lead sulfide, if dried and pulverized, reacts quickly with acid, neutral, and alkaline solutions of hydrogen peroxide. The conversion into sulfate is best seen if filter paper impregnated with lead sulfide is used. In this way, the quantity of lead sulfate can be kept so small that a white speck surrounded by gray appears when a drop of hydrogen peroxide is placed on the reagent paper.

Procedure: A drop of the slightly acid test solution is placed on dry filter paper impregnated with lead sulfide. This is then held over a heated asbestos mat for a short time. A white spot or ring appears on the black or gray paper, depending on the quantity of hydrogen peroxide or peroxide.

The lead sulfide paper is prepared as follows: Strips of filter paper (Schleicher and Schüll, 601; or Whatman, drop reaction paper 120) are moistened with 0.05 per cent (or more dilute) lead acetate solution, exposed to a current of hydrogen sulfide and dried in a vacuum desiccator. This reagent paper can be kept for a long time if stored in brown glass containers.

Identification Limit: 0.04 γ hydrogen peroxide.

Concentration Limit: 1:1,250,000.

Application in the presence of other materials: It should be noted in applying this test for hydrogen peroxide that lead sulfide is oxidized to lead sulfate in acid solution by all strong oxidizing agents (chlorate, perchlorate, bromate, iodate, periodate, nitrate). Only bromate, iodate, and periodate accomplish the oxidation in alkaline media.

11. Detection of Halide and Similar Ions

Three different procedures for preliminary tests for halide and similar ions will be given. They can also be used to distinguish between certain groups of these ions. The procedures described in (a) and (b) have simple chemical bases. In contrast, the procedure described in (c) involves not only chemical reactions, but also adsorption and photochemical processes which can be seen only if the test is carried out as a spot reaction on paper.

(a) Detection of Chloride, Bromide, Iodide, Cyanide, Thiocyanate, Ferricyanide

Chemical Basis: Reaction with silver ferrocyanide in the presence of ferric salt. White silver ferrocyanide is only slightly soluble in water, but it dissolves to a measurable extent. The dissolved portion, as is true in the case of all slightly soluble compounds, is in equilibrium with the solid phase, and also with the ions:

$$\underset{\text{(solid)}}{Ag_4Fe(CN)_6} \rightleftarrows \underset{\text{(dissolved)}}{Ag_4Fe(CN)_6} \rightleftarrows 4\,Ag^+ + Fe(CN)_6^{----}.$$

The equilibrium concentration of $Fe(CN)_6^{----}$ ions is so small that only a minute quantity of Prussian blue (ferric ferrocyanide) forms on the addi-

tion of a dilute ferric solution. If a ferric solution which has been treated with potassium fluoride is used no reaction at all ensues, because the concentration of ferric ions has been so greatly reduced through the formation of complex FeF_6^{---} ions, that they, together with the $Fe(CN)_6^{----}$ ions present in the solution, do not exceed the solubility product of Prussian blue. If, however, a mixture of $Ag_4Fe(CN)_6$ and FeF_6^{---} ions is treated with a halide whose silver salt is less soluble than silver ferrocyanide, the equilibrium Ag^+ ions will be withdrawn, and the concentration of $Fe(CN)_6^{----}$ ions thus raised to the extent that Prussian blue can be formed even with solutions containing FeF_6^{---} ions. This precipitation of Prussian blue through disturbance of the equilibrium can serve as a preliminary test to detect the presence of soluble chlorides, bromides, iodides, cyanides, thiocyanates, and ferricyanides.

Procedure: A little silver ferrocyanide suspension is placed in a depression of a spot plate and one drop of the acid or neutral test solution is then added, followed by one drop of ferric fluoride solution. The white silver salt turns blue immediately, if halide ion is present. If only minute quantities of halide are suspected, a droplet (the size of a pin head) of the silver ferrocyanide suspension should be used. It is best to confirm the test by a blank.

The *reagents* are prepared as follows. Silver ferrocyanide suspension: Silver nitrate solution is treated with excess potassium ferrocyanide; the precipitate is washed well by decantation, suspended in water, and a few drops of sulfuric acid and methyl orange added. The suspension is stable if stored in the dark.

Ferric fluoride solution: 1 per cent ferric nitrate solution is treated dropwise with concentrated potassium bifluoride solution until the yellow color is discharged.

	Cl^-	Br^-	I^-	$Fe(CN)^{----}$
Identification Limit:	3.0 γ	2.5 γ	2.0 γ	2.5 γ
Concentration Limit:	1:16,600	1:20,000	1:25,000	1:20,000

Application in the presence of other materials: The foregoing test merely indicates the presence of ions whose silver salts are less soluble than silver ferrocyanide. It does not distinguish between these ions. Azides, chlorates, bromates, iodates, perchlorates, and periodates do not react.

(b) Detection of Chloride, Bromide, Iodide, Cyanide, Thiocyanate, Azide, Ferrocyanide, Ferricyanide

Chemical Basis: Reaction with silver chromate. Silver chromate undergoes a double decomposition with halides (Hal):

$$Ag_2CrO_4 + 2\ Hal^- \rightarrow 2\ AgHal + CrO_4^{--}.$$

This reaction is easily detected by the disappearance of the red brown silver chromate if a drop of the halide solution is placed on filter paper im-

pregnated with silver chromate. A white spot or ring results. Paper of varying concentrations of silver chromate can be prepared; weakly impregnated papers are best for detecting small quantities of halide.

Procedure: Thick filter paper (Schleicher and Schüll, 601) is immersed in an ammoniacal solution of silver chromate, $[Ag(NH_3)_2]_2CrO_4$, the excess liquid drained off, and the paper dried in a current of warm air. Because of volatilization of the ammonia, very finely divided pure silver chromate is deposited in the capillaries of the paper on the side turned to the warm air. Silver chromate paper is not stable; when stored, particularly in direct daylight, partial reduction ensues, and is revealed by discoloration.

A drop of the neutral or acidified (acetic acid) test solution is placed on freshly prepared silver chromate paper. If considerable quantities of halide are present a white or yellow spot appears immediately; smaller quatities produce a smaller white ring.

The silver ammine chromate solution is prepared thus: 0.2 g. Ag_2CrO_4 is dissolved in concentrated ammonia and made up to 100 ml. with water.

Identification Limit:	Cl^-	Br^-	I^-	CNS^-
	2.5 γ	5.0 γ	5.0 γ	10.0 γ
Concentration Limit:	1:20,000	1:10,000	1:10,000	1:35,000

(c) **Detection of Chloride, Bromide, Iodide in the Presence of Other Halides**

Chemical Basis: Photolysis of silver halide and nuclear action. Certain silver halides (AgCl, AgBr, AgI) when exposed to the light are partially reduced and form violet to dark brown products that are known as photo- or subhalides of silver. The conversion of silver halide into a subhalide, which can be detected much more readily, occurs even in daylight. This photolysis proceeds more rapidly if the silver halide is finely divided and is exposed to the rays of a quartz lamp. Both of these conditions can be realized if the reaction is conducted as a spot test.

The silver halide, which is to be subjected to photolysis, is prepared best by procedure (b) that is by placing a drop of the test solution on silver chromate paper. The spot is held over ammonia water, and the silver chromate remaining in the zone moistened by the drop is thus converted into $[Ag(NH_3)_2]_2CrO_4$. Most of this is removed by washing with water, but a complete removal is not possible since the paper always retains some silver salt by adsorption. If the moist paper is now exposed to direct sunlight, or still better, to the rays of a quartz lamp, brown spots will appear in a few minutes at the place where silver halide was formed.

The activity of iodide and bromide is quite obvious since AgI and AgBr are insoluble or only slightly soluble in ammonia, and consequently, they remain on the paper after the treatment with ammonia and the washing, and are available for the photolysis. In contrast, the reason for the

similar behavior of silver chloride is not immediately apparent, since this salt is easily soluble in ammonia, and consequently should be removed when the paper is washed. This seemingly abnormal behavior can be explained as follows. When silver chloride is formed on the silver chromate paper in daylight, silver subchloride is also produced because of the fine state of division of the chloride. The subchloride, or the free silver contained in it, is not soluble in ammonia and remains after the paper is washed and, like AgBr and AgI, is further reduced under the quartz lamp. This explanation can be checked quite simply. If the spotting of the silver chromate paper (formation of AgCl) and the subsequent treatment with ammonia and washing are done in the dark room where no silver subchloride can form, not the slightest reduction occurs when the test spot is illuminated under the quartz lamp.

The reduction spots produced by ultraviolet light are not due solely to the reduction of silver halide. The photohalides formed by the photolysis of AgCl, AgBr, and AgI act also as nuclei for the reduction of the silver or silver-ammine ions adsorbed on the paper or on the silver halide. These centers thus accelerate the reduction of these ions in the ultraviolet light. An experimental proof of this action is provided if a drop of strong potassium bromide solution is placed on paper impregnated with only a little silver chromate. In this case all of the silver chromate in the immediate vicinity is consumed in the spot reaction. The action of ammonia, washing, and irradiation then results in no brown spot, or merely in a slight brown border at the extreme edges of the spot, that is, in the direct neighborhood of unreacted silver chromate.

Procedure: Filter paper (S. and S., 589) is soaked in a strong solution of silver-ammino-chromate, drained, and dried in a current of warm air. A drop of the neutral or slightly acid (acetic acid) test solution is placed on the brown paper and exposed to rays from the quartz lamp for one minute to convert part of the silver chloride to subchloride. The spot is then exposed to ammonia until all of the paper moistened by the liquid has become bright yellow (formation of silver-ammino-chromate). The paper is washed slightly with water, placed on a glass plate or slide and irradiated with a quartz lamp. If the sample contains chloride, bromide, or iodide, a brown spot or ring will appear within a few minutes.

The silver-ammino-chromate solution is prepared thus: 1 g. Ag_2CrO_4 is dissolved in concentrated ammonia and diluted to 100 ml. with water.

Identification Limit: 0.3 γ Cl^- or Br^- or I^-.

Concentration Limit: 1:167,000.

Application in the presence of other materials: Only silver chloride, bromide, and iodide are photochemically reduced under the foregoing experimental conditions. Consequently, these halides can be detected

even in the presence of other ions precipitable by silver ions. The activity of AgCl can be nullified, so that bromide and iodide can be detected in chlorides, if the spotting of the silver chromate paper, the treatment with ammonia and the washing are carried out in the dark room.

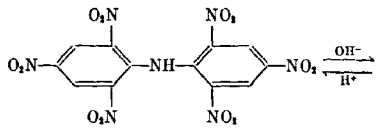
B. Spot Reactions with Organic Reagents

The use of organic reagents in spot test analysis involves, for the most part, the formation of mixed inorganic-organic reaction products. The organic compounds may participate in such reactions in three different ways. First, normal salts may be formed by inclusion of the inorganic material to be detected in the cationic or anionic part of the compound. Such salt formations involve principal valences; the reactions are useful for spot testing if the product is colored, or slightly soluble, or both. Organic reagents are used much more frequently, however, to combine with the material being sought to form a complex compound. In this type of reaction the ability of organic compounds to form inner complex compounds is particularly important. These compounds are salt-like products, in which a metal or an inorganic radical replaces an acid hydrogen, and in addition is bound to a second atom of the organic molecule by an auxiliary valence. Five or six membered ring systems are thus produced, and frequently they are responsible for the color and/or abnormal solubility relationships. The binding of an organic molecule through auxiliary valences alone, so that it becomes the neutral portion of the product (addition compound), can produce analogous effects. Adsorption compounds, in which the adsorption is accompanied by a deepening of the color of the adsorbed material, also belong in this category. The third valuable factor in the use of organic reagents is the ability of certain compounds to exhibit definite color changes when they are oxidized or reduced by inorganic materials. Frequently, even minimal quantities of the oxidizing or reducing agent can be detected in such cases.

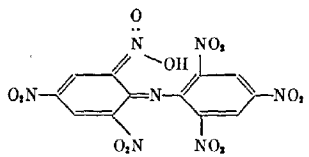
1. DIPICRYLAMINE

Dipicrylamine (hexanitro-diphenylamine) is a bright yellow crystalline compound, only slightly soluble in water and acids. The weak basic character of its parent compound, diphenylamine, has been completely dissipated by the entrance of six nitro groups and has been exchanged for an acidic character, which is exhibited in the solubility of dipicrylamine in sodium hydroxide or sodium carbonate solution. This salt formation, however, is not due to the replacement by sodium of the hydrogen atom of the imine group (=NH), but results from a preliminary rearrangement in which this hydrogen migrates and forms an $=NO_2H$ group. This functions as the real acidic group and forms the salts. The transformation of the yellow

"baso form" (I), which is insoluble in water, into the orange red soluble "aci form" (II), from which the salts are derived, is brought about by hydroxyl ions and can be reversed by hydrogen ions. Consequently, the tautomeric forms present the equilibrium:



(I)



(II)

It should be noted that the two forms of dipicrylamine differ by more than the development of an $=NO_2H$ group. The rearrangement of the molecule results in a chain of conjugated double bonds and also in an orthoquinoid linkage. The accumulation of conjugated double bonds and the ortho-quinone formation have a chromotropic (color intensifying) effect which is shown, in this case, by the change from yellow to orange red.

The orange red sodium salt has the aci structure (II). This is also true of all the other salts of dipicrylamine obtainable as orange-red precipitates by treating their solutions with a solution of the alkali salt (Na, K, Cs, Rb, Tl salt).

The solubility of dipicrylamine and its salts in aliphatic and aromatic nitro compounds (nitromethane, nitrobenzene, etc.) is noteworthy. This is an example of the heightened solubility frequently encountered when the solvent and solute are similar. The nitro groups are responsible for the effect in this instance.

Detection of Potassium

Chemical Basis: Formation of acid-stable potassium dipicrylamine. Orange-red, crystalline potassium dipicrylamine, slightly soluble in water, is produced as a precipitate by the action of a water solution of sodium dipicrylamine on neutral solutions of potassium salts. The potassium salt is not only less soluble than the sodium salt but is also more resistant to

dilute mineral acids. The sodium salt, in contrast, is decomposed by acids, with formation and precipitation of bright yellow dipicrylamine (baso-form). Despite the stability of the potassium salt toward dilute acids, it cannot be precipitated from an acid solution of dipicrylamine because the precipitation requires the presence of the aci form. This exists, however, only in the sodium salt, which is obtained by dissolving dipicrylamine in dilute sodium hydroxide or carbonate.

Procedure: A drop of the neutral test solution is placed on dry filter paper impregnated with sodium dipicrylamine and dried in a current of heated air. The orange reagent paper is then bathed in 0.1 *N* nitric acid until the paper turns bright yellow. A red spot or ring remains on the bright yellow paper if potassium is present. If small quantities of the latter are involved the paper should be placed in water after the acid treatment to avoid a too lengthy action of the acid.

Dipicrylamine paper is prepared by soaking strips of filter paper (S. and S., 598) for several minutes in a solution of sodium dipicrylamine. The solution is obtained by dissolving 0.2 g. dipicrylamine in 2 ml. of 2 *N* sodium carbonate, diluting with 15 ml. water and filtering. The excess solution is allowed to drain off and the paper is dried in a current of heated air applied to one side only. The paper can be kept for several weeks. The test solution should be placed on the more deeply colored side of the paper.

Identification Limit: 3 γ potassium.

Concentration Limit: 1:16,000.

Application in the presence of other materials: Cesium and rubidium react similarly to potassium, as do considerable quantities of ammonium salts. The latter, however, can be removed completely by igniting the test specimen. Small amounts of potassium cannot be detected by means of dipicrylamine if very large quantities of sodium are also present. Under these circumstances the solubility of sodium dipicrylamine is so low that it no longer reacts with potassium ion. In one drop, 10 γ potassium can be detected positively in the presence of 120 γ sodium. Although, with the exception of lead and mercury, di- and trivalent metals do not form insoluble dipicrylamine salts, the detection of small quantities of potassium fails in solutions of "indifferent" salts. The reason is that sodium dipicrylamine reacts alkaline because of hydrolysis:

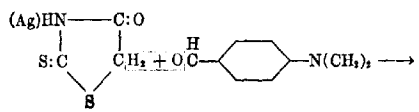
$$\mathrm{NaDip} + \mathrm{HOH} = \mathrm{NaOH} + \mathrm{HDip}$$

Consequently, the metal hydroxide is precipitated and the concentration of dipicrylamine ions is reduced to the point where no potassium salt is deposited. Therefore, if potassium is to be detected in the presence of zinc or other metal ions which can be thrown down as hydroxides, a mixture of the oxides and carbonates must be prepared as a preliminary step. A

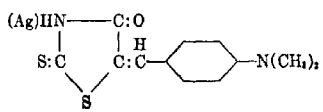
small quantity of this mixture is placed on the reagent paper, a drop of water added, dried in heated air, and the paper then bathed in 0.1 *N* nitric acid. This procedure permits the positive identification of 50 γ potassium in the presence of 5000 γ zinc. Nitrates can be changed into oxides or carbonates by evaporating the solution and igniting. Chlorides are converted into nitrates by repeated evaporation with concentrated nitric acid. Precipitation with barium nitrate is required in the case of sulfates.

2. *p*-DIMETHYLAMINO-BENZYLIDENE RHODANINE

Rhodanine (I) contains the silver binding —NH group. Consequently, it forms a white yellow silver salt, insoluble in acids or ammonia. The $-CH_2$ group of the rhodanine ring is very reactive. It easily undergoes condensation with aldehydes and ketones and then gives rise to numerous derivatives of rhodanine. The —NH group remains intact and retains its silver binding power in such condensation products. This made the synthesis of the first organic color reagent for silver possible. It is *p*-dimethylamino-benzylidene rhodanine (III), the condensation product of rhodanine and *p*-dimethylamino-benzaldehyde (II).



(I) (II)

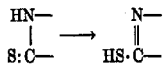


(III)

p-Dimethylamino-benzylidene rhodanine is a brown-yellow crystalline solid, slightly soluble in water and quite soluble in alcohol, acetone, and ether. The red-violet silver salt is insoluble in water and organic solvents, and is stable toward acids. It owes its color to the chromotropic effect of the $-N(CH_3)_2$ group.

p-Dimethylamino-benzylidene rhodanine in acid solution is not a specific but a selective reagent for silver, because it also forms violet precipitates with Cu^+, Hg^{++}, Pd^{++}, Pt^{++}, and Au^+. It loses its selective action in ammoniacal solution, and with numerous heavy metal ions it forms precipitates which decompose with formation of sulfides. The non-specificity in ammoniacal solution arises from the fact that the —NH

group is no longer present under such conditions. A tautomeric rearrangement



occurs, and produces the —SH group, which can react with all ions that can form sulfides.

Detection of Silver

Chemical Basis: Precipitation of silver benzylidene rhodanine. *p*-Dimethylamino-benzylidene rhodanine precipitates a red violet salt from weakly acid silver solutions. The precipitation is slow and incomplete from strongly acid solutions. The test can be carried out as a spot reaction on filter paper impregnated with the reagent or as a test tube reaction. The latter is recommended if considerable amounts of copper salts are present, and also when the silver must be detected at high dilutions.

Procedures: (*1*) Filter paper (S. and S., 601) is bathed in a saturated solution of the reagent in acetone and dried. A drop of the weakly acid test solution is applied. A red-violet precipitate or color appears, depending on the quantity of silver present. A positive result is easily seen even with minute quantities of silver, despite the yellow-brown color of the reagent. After the reaction has occurred, the filter paper can be bathed in acetone, which dissolves the unused reagent; the red-violet silver salt remaining behind on the paper.

(*2*) Several drops of the reagent solution are added to 1 to 5 ml. of the solution to be tested. Several minutes later the mixture is shaken with 1 or 2 ml. of ether. The unused reagent passes quantitatively into the ether layer, which becomes yellow; the silver salt forms a violet film at the water-ether interface and thus becomes easily visible.

Identification Limit: 0.02 γ silver in 0.05 ml. 1 γ silver in 5 ml.

Concentration Limit: 1:2,500,000 1:5,000,000

Application in the presence of other materials: In acid solution *p*-dimethylamino-benzylidene rhodanine also reacts with mercury, gold, platinum, and palladium salts.

The interference by mercury salts can be obviated as follows: the reaction is carried out on reagent paper, where both the mercury and the silver salt of the reagent are formed. The spot is then treated with several drops of dilute hydrochloric acid or ammonium chloride. These dissolve the mercury salt, forming slightly dissociated mercuric chloride, leaving the silver salt unchanged.

If silver is to be detected in the presence of gold, platinum, or palladium salts, a drop of the weakly acid test solution is placed on a spot plate

and mixed with one drop of 10 per cent potassium cyanide solution (formation of cyanides). One drop of reagent solution is then added, and the mixture acidified with dilute nitric acid (1:4). A pink coloration indicates the presence of silver. The test for silver by this cyanide method is valid only if copper is absent. Otherwise cuprous cyanide is formed which gives a reaction similar to the silver test with *p*-dimethylamino-benzylidene rhodanine.

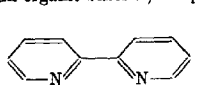
Demonstration of the Reduction of Cupric Salts by Filter Paper

It is well known that cuprous salts are quite similar to silver salts with regard to the formation of slightly soluble compounds. This has just been shown to be true also of the reaction with *p*-dimethylamino-benzylidene rhodanine. Consequently, this reagent can be used as a sensitive test for copper, if it is reduced to the cuprous state by treating the test solution with potassium cyanide or sulfur dioxide.

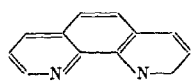
If a drop of neutral copper sulfate solution is allowed to stand in contact with filter paper for a short time reduction to cuprous salt occurs. Subsequent spotting with *p*-dimethylamino-benzylidene rhodanine and bathing in acetone leaves a red fleck of cuprous benzylidene rhodanine on the paper. The spot is more intense the longer the copper solution has been in contact with the paper; gentle warming also accelerates the reduction. It is well to run a parallel experiment on a spot plate with one drop each of the copper solution and of the reagent. A neutral copper solution that has not been in contact with paper does not react with *p*-dimethylamino-benzylidene rhodanine.

3. α,α'-DIPYRIDYL AND α,α'-PHENANTHROLINE

The weak organic bases α,α'-dipyridyl (I) and α,α'-phenanthroline (II)



(I, α, α'-Dip)

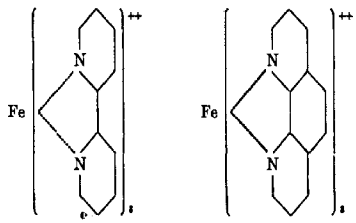


(II, α, α'-Phen)

are only slightly soluble in water but dissolve easily in dilute acids, forming the corresponding salts. Solutions of the chlorides or sulfates are of interest for spot testing because ferrous ions form extremely stable deep red complex cations with three molecules of either (I) or (II). These cations are stable toward dilute acids, alkalies and, in the cold, even ammonium sulfide. Hence, even acid ferrous solutions react with the chloride or sulfate of these bases. The stability and color of the complex ions is due to the five membered rings formed through auxiliary valences.

Ferrous dipyridyl and ferrous phenanthroline ions can be formulated as

hexammine ions, in which dipyridyl or phenanthroline occupy six coordination places on the iron atom:



$Fe(\alpha,\alpha'\text{-Dip})_3^{++}$ $Fe(\alpha,\alpha'\text{-Phen})_3^{++}$

Ferric ions form no analogous complex ions by direct action. The corresponding soluble blue ferric complex ions can be obtained only by oxidation of the previously prepared ferrous compounds. The ferric complexes, however, in contrast to the ferrous compounds, are unstable. Even traces of reducing agents suffice to regenerate the stable red ferrous compounds.

The great volume or high atomicity of ferrous dipyridyl or ferrous phenanthroline ions make them good precipitants for numerous complex ions, such as complex cyanides, iodides, thiocyanates, and so forth. Red crystalline compounds, for example

$$[Fe(\alpha,\alpha'\text{-Dip})_3][Ni(CN)_4], \quad [Fe(\alpha,\alpha'\text{-Dip})_3][HgI_4],$$

etc., are produced. The precipitation of CdI_4^{--} by $Fe(\alpha,\alpha'\text{-Dip})_3^{++}$ under controlled conditions makes it possible to detect cadmium with certainty.

Detection of Iron (and Reducing Compounds)

Chemical Basis: Formation of red ferrous salt of α,α'-dipyridyl (phenanthroline). Red complex cations of the hexammine ion type are formed in mineral acid solution by ferrous ions with α,α'-dipyridyl or α,α'-phenanthroline. Ferric ions do not react with these bases. Consequently, ferrous iron can be detected in the presence of trivalent iron. If iron is to be detected without reference to its valence, preliminary reduction of the test solution with sulfur dioxide is essential.

The following method is much more sensitive than the usual test for iron with thiocyanate. It has the further advantage that it can be applied in the presence of materials which interfere with the thiocyanate reaction (fluorides, phosphates, etc.).

The fact that ferric salts do not react with these bases can be utilized to detect materials which reduce ferric salts. If stannous chloride, sulfur dioxide, etc., are added to a yellow acid solution containing a ferric salt and

α,α'-dipyridyl (phenanthroline), the ferrous salt is formed and is revealed immediately by the development of the red complex ion.

Procedure: One drop of the acid test solution is treated on a spot plate with one drop of a 2 per cent solution of the base in dilute hydrochloric acid. The test may also be carried out on filter paper impregnated with a two per cent solution of the reagent in alcohol and then dried. A pink to red color develops, according to the quantity of iron present. Similarly colored circles or rings are formed on the paper.

Identification Limit: 0.03 γ iron.

Concentration Limit: 1:1,660,000.

Application in the presence of other materials: The test for iron with α,α'-dipyridyl or α,α'-phenanthroline is specific. It is true that other ammine-forming ions (Cu^{++}, Zn^{++}, Cd^{++}, etc.) likewise react with these two bases, in weakly acid solutions, but the corresponding ammine ions, in contrast to the ferrous ammine ion, are not highly colored. Therefore, if sufficient quantities of the reagent are used, there is no interference with the test for iron. Although very large quantities of halide or sulfate ions decrease the solubility of the corresponding ferrous dipyridyl (phenanthroline) compounds in water so that, under such circumstances, a red precipitate instead of a red coloration ensues, no real interference results. It should be noted, however, that the recognition of small quantities of the red ferrous complex compound is more difficult in highly colored solutions. It is then necessary to carry out a blank (comparison) test, or the solution under investigation is treated first with α,α'-dipyridyl (phenanthroline) solution and then with a drop of a solution of K_2CdI_4. Red $[Fe(\alpha,\alpha'\text{-Dip})_3][CdI_4]$ will precipitate (see p. 217) and can be collected by centrifuging and thus be made more visible. The whole operation can be carried out with one drop of test solution in a microcentrifuge tube. The K_2CdI_4 solution is prepared by adding an excess of potassium iodide solution to a solution of a cadmium salt.

Detection of Cadmium

Chemical Basis: Precipitation of red ferrous-α,α'-dipyridyl-cadmium iodide. Red solutions of ferrous α,α'-dipyridyl salts, or the $[Fe(\alpha,\alpha'\text{-Dip})_3]^{++}$ ions contained in them, react with numerous complex anions to form red crystalline precipitates. The precipitability of complex cadmium iodide by this means is utilized in the present test. Cd^{++} ions combine with excess I^- ions:

$$Cd^{++} + 4I^- \rightleftharpoons CdI_4^{--}$$

The complex cadmium iodide ion then combines with ferrous α,α'-dipyridyl ion and forms a red crystalline precipitate:

$$CdI_4^{--} + Fe(\alpha,\alpha'\text{-Dip})_3^{++} \rightarrow [Fe(\alpha,\alpha'\text{-Dip})_3][CdI_4].$$

In the practical application of this precipitation reaction it is advantageous to prepare a reagent solution containing $[Fe(\alpha,\alpha'\text{-Dip})_3]^{++}$ ions and excess iodide ions. This solution contains all the ionic species required to react with cadmium ions to produce the foregoing complex compound:

$$Cd^{++} + 4I^- + [Fe(\alpha,\alpha'\text{-Dip})_3]^{++} \rightarrow [Fe(\alpha,\alpha'\text{-Dip})_3][CdI_4].$$

The precipitation of cadmium is complete from weakly acid, neutral, or ammoniacal solution.

Procedure: A drop of the reagent solution is placed on spot paper and, before it has a chance to be soaked up, a drop of the test solution is placed directly on the first drop. The reaction occurs at once. After the absorption has taken place a spot or ring remains. Because of its intense red color, it can be distinguished easily from the red reagent solution retained in the paper. A blank test is recommended only when very small quantities of cadmium are suspected.

The solutions can be brought together in a microcentrifuge tube. It is best to introduce a drop of the reagent into the tube first, followed by a drop of the test solution. The drops should not be mixed. On centrifuging, a red precipitate will be produced in the constricted end of the tube. Strangely enough, if the drops are mixed before centrifuging, the sensitivity of the test is considerably decreased.

The $[Fe(\alpha,\alpha'\text{-Dip})_3]I_2$ solution is prepared by dissolving 0.25 g. α,α'-dipyridyl and 0.146 g. $FeSO_4 \cdot 7H_2O$ in 50 ml. water, adding 10 g. KI, shaking thoroughly, and filtering. The solution keeps. In case it becomes turbid it must be filtered before using.

Identification Limit: 0.05 γ cadmium.

Concentration Limit: 1:1,000,000.

Application in the presence of other materials: The direct use of $[Fe(\alpha,\alpha'\text{-Dip})_3]I_2$ as a precipitant for cadmium is interfered with by metal ions which can form slightly soluble or complex iodides. Solutions of silver, copper, or lead salts form precipitates with the iodide contained in the reagent, and these precipitates are then colored pink by adsorption of $[Fe(\alpha,\alpha'\text{-Dip})_3]^{++}$ ions. Cupric salts, in addition, liberate iodine from neutral or acid solutions. Bismuth, mercury, tin, and antimony salts form soluble complex iodides which likewise combine with $[Fe(\alpha,\alpha'\text{-Dip})_3]^{++}$ ions to form red precipitates. These latter reactions, however, are not so sensitive as the cadmium reaction. The test for cadmium can be rendered almost specific, despite the hindrance of iodide-forming ions, if the test solution is first treated with ammonia, which precipitates lead, bismuth, mercury, tin, and antimony as hydrated oxides. The ammoniacal filtrate is then tested with the reagent. The $Cd(NH_3)_4^{++}$ ions in the filtrate react promptly, whereas the $Cu(NH_3)_4^{++}$ ions are inactive. Only $Ag(NH_3)_2^+$ ions, which may likewise appear in the filtrate, can interfere, as they form silver iodide with the

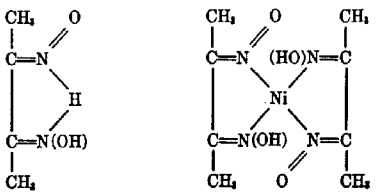
iodide in the reagent. If, therefore, cadmium is to be detected in the presence of silver and other iodide-forming ions, the following procedure should be used. The test solution is treated with dilute hydrochloric acid and any precipitate removed by filtration. An excess of ammonia water is added, any precipitate filtered off, and the ammoniacal filtrate then tested with the reagent. One or two drops of a test solution are sufficient for all these operations. They can be done in microcentrifuge tube in which the precipitate can easily be separated by centrifuging.

4. DIMETHYLGLYOXIME

All 1,2-diketones, $R\cdot C(:O)\cdot C(:O)\cdot R'$, can be converted into dioximes $R\cdot C(:N\cdot OH)—C(:N\cdot OH)\cdot R'$. These may occur in three forms (syn-, amphi-, anti-), which differ in the geometric orientation of the $=N\cdot OH$ groups.

All anti-dioximes, $\begin{matrix} R—C—C—R \\ \quad\| \quad\| \\ HO\cdot N \quad N\cdot OH \end{matrix}$ react with ammoniacal or acetic acid solutions of nickel salts, red or red yellow crystalline inner complex salts being precipitated. This occurs even at high dilutions. As the nature of the radicals R and R' has no influence, the activity of these reagents is due to the grouping $\begin{matrix} —C—C— \\ \quad\| \quad\| \\ HO\cdot N \quad N\cdot OH \end{matrix}$.

The dioxime of diacetyl is more frequently used in chemical analysis than any other compound of this type. It is usually called dimethylglyoxime. This white crystalline compound is only slightly soluble in water, alkalies, and acids; saturated (about 1 per cent) alcoholic solutions are used as reagent. Dimethylglyoxime, like all other 1,2-dioximes, functions as a monobasic acid when the nickel salt is formed, that is, the hydrogen atom of only one $=N\cdot OH$ group can be replaced by the metal. Recent investigations have shown that the —NOH groups of oximes react in the tautomeric $=N\begin{matrix} \diagup O \\ \diagdown H \end{matrix}$ form when the salts are formed. Accordingly, dimethylglyoxime itself, as well as its nickel salt, can be written as an inner complex compound, in which the acid hydrogen and the metal are part of a five-membered ring linked through principal and auxiliary valences:



Only palladium forms a salt whose structure is like that of the nickel compound. However, yellow palladium dimethylglyoxime, in contrast to the nickel salt, is insoluble in acids and soluble in ammonia. Consequently, the precipitation of nickel with dimethylglyoxime from an ammoniacal solution is specific. It should be noted that ferrous-, copper-, and cobalt salts form colored soluble complex compounds with dimethylglyoxime in ammoniacal solution; these products do not have the structure of the nickel salt.

Detection of Nickel

Chemical Basis: Precipitation of red nickel dimethylglyoxime. Dimethylglyoxime produces a red precipitate of inner complex nickel dimethylglyoxime from neutral, acetic, or ammoniacal solutions. This salt is so insoluble in water that even nickel oxide, carbonate, cyanide, and other very slightly soluble nickel compounds react with this reagent.

The test is very sensitive and quite selective. However, the visibility of the nickel dimethylglyoxime precipitate is very much lower in the presence of considerable quantities of materials which produce colored or slightly soluble compounds in ammoniacal solutions (Fe^{+++}, Co^{++}, Cu^{++}, etc.). Consequently, under such circumstances, special measures are necessary in detecting small amounts of nickel.

Procedure: Filter paper is impregnated with a saturated alcoholic solution of dimethylglyoxime and dried. A drop of the acid or neutral test solution is placed on the freshly prepared paper. Any free acid is removed by holding the spot over 6 *N* ammonia. The formation of a red fleck or ring is noted.

Identification Limit: 0.015 γ nickel.

Concentration Limit: 1:3,600,000.

Application in the presence of other materials: The addition of dimethylglyoxime to solutions of cobalt salts produces no precipitate because no cobalt salt analogous to the nickel compound exists. However, a yellow to brown color immediately appears, due to formation of soluble complex compounds of di- and trivalent cobalt. As dimethylglyoxime has a rather limited solubility (in alcohol about 1 per cent), solutions containing little nickel in the presence of much cobalt will not produce the nickel compound, because all the reagent is consumed by the cobalt. Under certain conditions, however (masking of the cobalt), it is possible to detect, with certainty, even traces of nickel in the presence of cobalt. The procedure, described in detail on p. 221, can also be used, in its essentials, if nickel is to be detected in solutions containing both cobalt and iron.

Although neither ferric nor cobalt salts form insoluble compounds with dimethylglyoxime when they are present separately, solutions containing

both of them produce a red-brown precipitate. This is a complex compound of iron, cobalt, and dimethylglyoxime; its formation can interfere with the detection of small amounts of nickel. However, this interference can be obviated by carrying out the test as follows: One or two drops of the test solution are warmed in a microcentrifuge tube with a solution of potassium cyanide until the precipitate which first forms has dissolved. A few milligrams of solid dimethylglyoxime and a few drops of formaldehyde solution are then added. The contents of the tube are stirred with a fine glass rod, and then centrifuged. If nickel is present, a red deposit appears.

Detection of Nickel in the Presence of Iron

As brown hydrated ferric oxide will conceal the nickel dimethylglyoxime precipitated in an alkaline medium, it is necessary to prevent the precipitation of iron. An alkali tartrate is used to mask the iron. One drop each of the test solution, of saturated sodium tartrate, and of alcoholic dimethylglyoxime are placed successively on a spot plate. If nickel is present, a red precipitate appears, which, because of the low surface tension of the alcohol, creeps to the upper edge of the liquid.

Identification Limit: 0.5 γ nickel } in presence of 1000 times
Concentration Limit: 1:100,000 } as much iron

Detection of Nickel in the Presence of Copper, Cobalt, or Manganese

Dimethylglyoxime reacts with copper or cobalt salts in ammoniacal solution to produce a brown soluble complex compound of copper or cobalt. Manganese salts, under these conditions, are converted partially to manganese dioxide. These reactions interfere with the perception of small quantities of nickel dimethylglyoxime. Hence, if small amounts of nickel are to be detected in the presence of copper, cobalt, or managanese, the following method is recommended. The test solution should be made as nearly neutral as possible. A drop is then placed on dimethylglyoxime paper. After the drop has been soaked up, the paper is bathed in dilute ammonia. The colored dimethylglyoxime compounds of copper and cobalt dissolve; a pink fleck remains on the white paper if nickel is present. The paper should be bathed in ammonium carbonate if manganese is present.

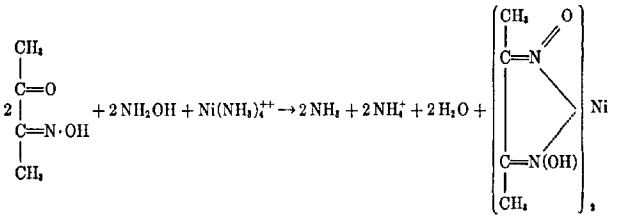
Identification Limit: 0.8 γ nickel } in the presence of 1250 times as
Concentration Limit: 1:63,000 } much cobalt

Identification Limit: 1.7 γ nickel } in the presence of 600 times as much
Concentration Limit: 1:30,000 } copper

Identification Limit: 0.1 γ nickel } in the presence of 10,000 times as
Concentration Limit: 1:500,000 } much manganese.

Detection of Hydroxylamine

Chemical Basis: Formation of nickel dimethylglyoxime from diacetylmonoxime, nickel salt, and hydroxylamine. An alkaline solution of hydroxylamine reacts with diacetylmonoxime to produce dimethylglyoxime. If this reaction occurs in the presence of a nickel salt the product will immediately function and form nickel dimethylglyoxime. Consequently, an ammoniacal solution of diacetylmonoxime containing nickel can act as a reagent for hydroxylamine:



It is well to saturate the reagent solution (ketone plus ammoniacal nickel solution) with nickel dimethylglyoxime to accelerate the deposition of the reaction product.

Procedure: The reagent is prepared by dissolving 1.2 g. diacetylmonoxime and 0.95 g. $NiCl_2 \cdot 6H_2O$ in 35 ml. hot water. After cooling, 2 ml. concentrated ammonia water is added. The solution is poured into 200 ml. of water containing 0.12 g. hydroxylamine and allowed to stand for a day. The precipitate of nickel dimethylglyoxime is filtered off and the red-brown filtrate used to prepare the paper. Filter paper is impregnated with the reagent solution and dried. A drop of the test solution is placed on the yellow paper. A more or less intense red fleck or ring of nickel dimethylglyoxime appears according to the quantity of hydroxylamine present.

Identification Limit: 1 γ hydroxylamine.

Concentration Limit: 1:50,000.

Application in the presence of other materials: As a rule, only alkalies, ammonium salts, or hydrazine need be considered when testing for hydroxylamine. The first two do not interfere. Large quantities of hydrazine react with the reagent solution and form a red brown precipitate. Nevertheless, the method as described will serve for the detection of 5 γ hydroxylamine in the presence of 300 γ hydrazine.

Detection of Palladium

Chemical Basis: Protective layer effect following reaction of nickel dimethylglyoxime with a palladium salt. If a drop of a neutral solution containing palladium is placed on paper impregnated with nickel dimethylglyoxime,

the latter, being finely dispersed in the capillaries of the paper, will react on the surface to produce palladium dimethylglyoxime. This compound is not soluble in acids and is able to protect any intermixed unchanged nickel dimethylglyoxime from attack by dilute acid. Even minimal quantities of palladium dimethylglyoxime, which of themselves cannot be observed, suffice for this protective action. As a consequence, after bathing in dilute acid, a red fleck will remain at the spot where a drop of a dilute palladium solution has been placed on red nickel dimethylglyoxime paper. The rest of the paper will become perfectly colorless. The reagent paper is prepared as described on p. 87.

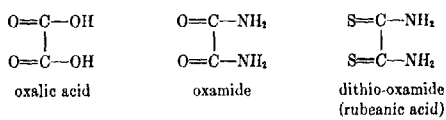
Identification Limit: 0.01 γ palladium.

Concentration Limit: 1:5,000,000.

Application in the presence of other materials: As only palladium salts undergo this double decomposition with nickel dimethylglyoxime, the test just described is specific. It is essential to use a neutral palladium solution, preferably of the nitrate.

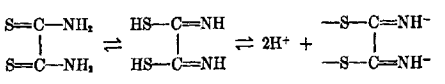
5. DITHIO-OXAMIDE (RUBEANIC ACID)

If the hydroxyl groups of oxalic acid are replaced by amino groups, oxamide results. The sulfur analog of oxamide, dithio-oxamide, is also known as rubeanic acid.

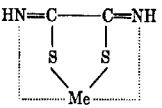


Rubeanic acid forms yellow-red crystals, which, in contrast to colorless oxamide, are soluble in alcohol, acetone, as well as in warm alkaline solutions. Consequently, the replacement of oxygen by sulfur in oxamide has not only a chromotropic effect (that is, the color is deepened) but the compound also becomes acidic as revealed by its solubility in alkalies. This acidifying effect of sulfur is often observed if oxygen compounds are compared with their sulfur analogs. Examples are H_2O—H_2S; phenol—thiophenol.

Dithio-oxamide can act as an acid because tautomeric changes in the molecule can result in the development of acidic —SH groups. In alcoholic solution there is an equilibrium between the amide form and a sulfhydryl (aci) form:



In its aci form, dithio-oxamide produces colored precipitates with several heavy metals. These can be viewed as inner complex compounds formed by binding the metal atom to the nitrogen atoms of the imid groups by auxiliary valences: The behavior of the ammine-forming ions (Ag^+, Cu^{++}, Cd^{++}, Zn^{++}, Ni^{++}, Co^{++}) is of particular analytical interest. Of these, only the colored ions (Cu^{++}, Co^{++}, Ni^{++}) react. The rubeanates of these metals can be written:



Me = copper, nickel, cobalt

These inner complex compounds are formed if the concentration of the aci-form of dithio-oxamide is so great that the solubility product of the particular rubeanate is exceeded. This occurs when the H^+ ion concentration is decreased by the presence of an alkali acetate, or by adding ammonia. It is noteworthy that after the rubeanates have once been precipitated they are insoluble in dilute mineral acids. This agrees with the concept that they are inner complex salts.

Detection of Copper, Cobalt, and Nickel

Chemical Basis: Precipitation of colored rubeanates. An alcoholic solution of rubeanic acid precipitates the corresponding copper (black)-, cobalt (brown)-, nickel (violet) salt. The precipitation is due to the fact that an alcoholic solution of the reagent contains an equilibrium mixture of the tautomeric amide- and aci-forms. The latter forms inner complex salts of copper, cobalt, nickel. The copper salt, because of its slight solubility, can be precipitated directly from an acetic acid solution. Complete precipitation of cobalt and nickel rubeanates demands, however, a higher concentration of the aci-form of the reagent, or removal of the H^+ ions. Both of these requirements can be effected by adding ammonia.

Procedure: A drop of the test solution, which should be as nearly neutral as possible, is placed on filter paper, held above ammonia water, and then treated with a drop of 1 per cent solution of rubeanic acid in alcohol. A black, brown, or violet fleck or ring results, according to the quantity of copper, cobalt, or nickel present. The following values for the sensitivity of the tests are obtained, if the spot reaction is made with one microdrop (0.015 ml.) of the test solution:

Identification Limit:	0.006 γ Cu	0.003 γ Co	0.012 γ Ni
Concentration Limit:	1:2,500,000	1:1,660,000	1:1,250,000

Detection of Copper in the Presence of Nickel and Cobalt by Capillary Separation

Copper rubeanate is the least soluble of these salts and its solubility product is also the lowest. Thus, the slight concentration of the aci-form of rubeanic acid provided by an alcoholic solution of the reagent suffices to attain this value, even though small quantities only of copper are present, and in an acidified (acetic) solution. Under these same conditions, the nickel and cobalt salts either do not precipitate at all, or do so incompletely.

In practice, the nickel or cobalt content of a test solution will not be known, and consequently, it is impossible to judge the proper quantity of acetic acid to be added. Therefore, this sensitive test for copper in the presence of cobalt and nickel cannot be carried out in a test tube without danger of coprecipitating some cobalt or nickel. However, if the test is made on filter paper, small quantities of copper can be detected easily by capillary separation, even though large amounts of cobalt and nickel are present.

Procedure: A drop of the test solution, acidified with acetic acid, is placed on filter paper impregnated with rubeanic acid. Two zones of differing acetic acid content are formed. The acid concentration is higher in the central zone and here copper alone is precipitated and forms an olive green or black circle. The nickel diffuses further and forms a blue violet ring around the central zone and builds up toward the middle as the acetic acid evaporates. The same process occurs in the presence of cobalt, except that the central zone is surrounded by a yellow-brown ring of cobalt rubeanate. If both cobalt and nickel are present, it is still possible to detect the copper by this method, provided the test solution does not contain more than two per cent of cobalt or nickel.

Identification Limit: 0.05 γ Copper
Concentration Limit: 1:1,000,000
} in the presence of 20,000 times as much nickel

Identification Limit: 0.25 γ Copper
Concentration Limit: 1:200,000
} in the presence of 2000 times as much cobalt (in acetic acid solution)

When only cobalt is present along with the copper, a drop of the neutral test solution may be placed on paper impregnated with rubeanic acid. A central ring of copper rubeanate will then be surrounded by a concentric brown yellow ring of cobalt rubeanate.

Identification Limit: 0.05 γ copper
Concentration Limit: 1:1,000,000
} in the presence of 20,000 times as much cobalt.

Detection of Nickel in the Presence of Cobalt, Iron, or Copper by Capillary Separation of the Ammine Salts

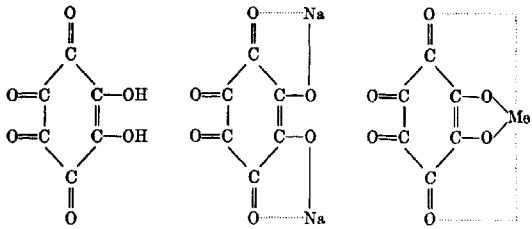
Copper, cobalt, and nickel are completely coprecipitated from an ammoniacal solution by rubeanic acid. If ferric salts are present, hydrated ferric oxide will be precipitated and interferes with the recognition of small quantities of nickel rubeanate. Nonetheless, within certain concentration limits, nickel can be detected in the presence of these metals, by utilizing the diverse diffusion velocities of their ammine salts in thin paper. The diffusion velocity of the nickel ammine salt is higher than that of cobalt or copper. Consequently, if a drop of an ammoniacal solution of these three salts is placed on paper, or if a drop of the neutral solution on paper is held over ammonia, the nickel diffuses to the outer zone of the spot. If a drop of the alcoholic reagent solution is placed at the side, it spreads, and a blue ring of nickel rubeanate surrounding a brown-green or brown circle of the corresponding cobalt and copper rubeanates is formed. A drop (0.15 ml.) of a solution containing 1 per cent of cobalt and 0.002 per cent nickel still shows a recognizable blue fringe around the yellow brown precipitation zone of the cobalt compound. This corresponds to an identification limit of 0.32 γ nickel in a limiting proportion of Ni:Co = 1:480.

By this method it is possible to detect 0.032 γ nickel in the presence of 4800 times as much iron.

6. RHODIZONIC ACID

Rhodizonic acid (I) is always used in the form of its sodium salt (II). This dark brown solid forms yellow to dark red yellow solutions according to the concentration. The acid and its alkali salts are easily oxidized and solutions of the reagent do not keep long. They are gradually oxidized by the air, the color fades, and carbon dioxide is evolved. The solutions can be kept for about five days if stored in the refrigerator. The decomposition of sodium rhodizonate by ultraviolet light is striking. If one drop of the dilute solution is placed on a spot plate and another on filter paper, and these are then irradiated under the quartz lamp, the yellow spot on the paper will be decolorized within a few minutes, whereas the drop on the spot plate loses its color only after about thirty minutes exposure. The high dispersion of the salt in the paper is responsible for the difference.

The color of sodium rhodizonate solutions is discharged on the addition of dilute mineral acids and of acetic acid; alkalization will restore the color. This fact shows that the yellow is not due to the rhodizonate ion, but is probably the color of the undissociated sodium salt, which can be regarded as an inner complex compound (II).



(I) (II) (III)

Sodium rhodizonate reacts with numerous uni- and divalent metal ions in neutral solution and forms colored precipitates. Typical instances are: Tl^{+} (black); Hg^{+} (brown-red); Hg^{++} (red-orange); Sn^{++} (violet); Cd^{++} (brown-yellow); Co^{++} (olive-green); Mn^{++} (dark brown); Pb^{++} (dark violet). These statements can be confirmed by placing a drop of the respective metal solutions in the depressions of a spot plate, adding a little ammonia water, and then a drop of sodium rhodizonate solution. Tri- and quadrivalent metal ions form no precipitate with sodium rhodizonate. Bismuth is an exception, probably because its solutions contain the BiO^{+} ion.

Table I (page 132) exhibits the findings obtained with 1 per cent neutral and acidified salt solutions. The behavior of the respective hydroxides and oxides is also recorded.

The behavior of iron salts is to be noted. Fe^{++} salts in neutral solution form a red-brown precipitate, which quickly becomes black-blue, probably by oxidation. In solutions of pH 2.8 there is no reaction. Fe^{+++} salts form no precipitate but produce a blue-green color; rhodizonic acid is a phenol and therefore reacts with Fe^{+++} salts like other phenols. In the presence of fluoride ion no color reaction occurs, owing to the formation of the complex FeF_6^{---} ion.

In the presence of fluoride ion the behavior of Fe^{++} salts is remarkable. The yellow of the rhodizonate solution is immediately discharged. No explanation can be given for this phenomenon, though it may be due to the increase of the reduction potential of Fe^{++} salts in the presence of fluoride ion, so that the oxidation of rhodizonic acid is induced by autoxidation of Fe^{++} salt.

The intense color of the insoluble rhodizonates indicates that they are to be viewed as inner complex salts of a polyketone as shown in (III). The color is due to the presence of a five-membered ring, in which the metal atom is coordinated by auxiliary valence linkages to the oxygens of the adjacent CO groups.

From the analytical standpoint it is particularly important that in the alkaline earth and alkali groups brown-red precipitates of the rhodizonate are produced only by Ba^{++} and Sr^{++}. However, under suitable conditions, these ions can be identified positively in the presence of each other.

The solubilities of lead rhodizonate (blue-violet or scarlet red) and barium rhodizonate are particularly low, even the oxides and carbonates of these metals reacting with sodium rhodizonate. Lead sulfate also gives a posi-

TABLE I

Reactions with Sodium Rhodizonate

Ion	*Salt Solution*		*Hydroxide*	*Oxide*
	Neutral	*pH = 2.8*		
Ag^{+}	Black	Black		
Hg^{+}	Brown-red	Brown-red (disappears on standing)		
Tl^{+}	Dark brown	Dark brown		
Pb^{++}	Blue-violet	Scarlet	Blue-violet	Blue-violet
Cu^{++}	Orange-red			
Hg^{++}	Red-orange			
Cd^{++}	Brown-red	Brown-red	Gray-brown	
Bi^{++}	Brown-red		Brown-red	
Ni^{++}				
Co^{++}				
Zn^{++}	Brown-violet		Brown-violet	Brown-violet
Mn^{++}				
UO_2^{++}	Brown			
Be^{++}				
Mg^{++}				
Ca^{++}			Brown-red	Brown-red
Ba^{++}	Red-brown	Red-brown	Red-brown	Red-brown
Sr^{++}	Red-brown		Red-brown	Red-brown
Sn^{++}		Violet	Violet	
Sn^{++++}				
Al^{+++}				
Zr^{++++}				

tive reaction. Methods for distinguishing these metals are based on these facts. Red-brown barium rhodizonate reacts with sulfate ion to form barium sulfate. A test for sulfate depends on this reaction.

Detection of Barium and Strontium

Chemical Basis: Precipitation of red-brown barium or strontium rhodizonate. Brown-red amorphous precipitates are produced when sodium rhodizonate is added to neutral solutions of barium or strontium salts. The addition of dilute acetic acid changes the color of these precipitates toward red,

probably by the formation of acid salts. Mineral acids dissolve the rhodizonates. However, the barium salt is much less soluble in dilute hydrochloric acid than the strontium salt.

Barium carbonate becomes brown almost immediately on contact with sodium rhodizonate. The transformation is incomplete, occurring only on the surface. Strontium carbonate does not react with sodium rhodizonate. If the carbonates have been precipitated together, the mixture fails to respond to this differentiating reaction, even at low limiting proportions, because the barium carbonate is coated by the inactive strontium carbonate.

Procedure: A drop of the neutral or slightly acid test solution is placed on filter paper and treated with a drop of a water solution (2 per cent) of sodium rhodizonate. A more or less intense red-brown fleck appears according to the quantity of barium or strontium present. It is also feasible to impregnate filter paper with sodium rhodizonate solution. The reagent paper must be dried in vacuo (over conc. H_2SO_4) and in the dark to avoid an oxidative deterioration of the reagent. A drop of neutral test solution will cause precipitation of the rhodizonate when placed on this reagent paper.

Identification Limit: 0.25 γ barium 0.45 γ strontium

Concentration Limit: 1:200,000 1:125,000

Application in the presence of other materials: Since numerous divalent metals produce colored rhodizonates, it is well to use sodium rhodizonate for the detection of barium and strontium within the alkaline earth and alkali groups. Beryllium, zinc, aluminum, and chromium may be present as they do not react with sodium rhodizonate. Large quantities of ammonium salts interfere; dark brown ammonium rhodizonate is deposited, but this disappears on addition of water.

A precipitate produced by ammonium carbonate can be tested for barium and strontium by the following procedure. The precipitate is washed thoroughly and a portion (size of a pin head) is treated on a spot plate with a drop of the rhodizonate solution and then with a drop of 6 *N* acetic acid. The presence of barium or strontium, or both, is revealed by the formation of a red precipitate.

Detection of Lead

Chemical Basis: Formation of lead rhodizonate. Neutral solutions of lead salts react with sodium rhodizonate to produce a blue-violet amorphous precipitate, $PbC_6O_6 \cdot Pb(OH)_2 \cdot 5H_2O$. This does not dissolve in dilute mineral acids nor in acetic acid, but these acids convert it into a less basic, brilliant red, micro-crystalline product, $2PbC_6O_6 \cdot Pb(OH)_2 \cdot H_2O$. If the lead solution is brought to pH = *ca.* 3 by means of a suitable buffer the red precipitate is obtained directly.

Procedure: A drop of the test solution is placed on filter paper and allowed to soak in. The spot is then touched with a drop of 0.2 per cent solution of sodium rhodizonate. A blue-violet fleck forms if lead is present.

Identification Limit: 0.1 γ lead.

Concentration Limit: 1:2,000,000.

The lead in extremely dilute solutions can be concentrated by the following procedure:

Ten milliliters of the test solution are treated with 1 ml. of 0.2 *N* mercuric chloride, the mixture acidified, and the mercury, together with the lead, then precipitated by hydrogen sulfide (not ammonium sulfide). The precipitate is collected by filtering or centrifuging, washed slightly, dried, separated from the filter paper, placed in a crucible, carefully heated at first, and then ignited to volatilize the mercuric sulfide. The cooled residue is treated with 3 drops of buffer solution and mixed, and then 1 drop of 0.2 per cent freshly prepared solution of sodium rhodizonate is added. If lead is present, a red precipitate or coloration will appear. The buffer solution (pH = 2.8) contains 15 g. tartaric acid and 19 g. sodium bitartrate per liter.

Identification Limit: 5 γ lead in 10 ml.

Concentration Limit: 1:2,000,000.

Application in the presence of other materials: Table I shows that only thallium, silver, cadmium, barium, and stannous tin also react at pH = 2.8. However, if certain conditions are maintained, it is possible to detect lead with certainty, even though these interfering ions are present.

In the presence of insoluble chlorides: Lead can easily be identified in the ordinary qualitative scheme when it is present along with silver, mercurous, and thallous chlorides.

The chlorides thrown down by hydrochloric acid are not washed but are transferred directly to a crucible, dried by gentle warming, and then carefully heated to redness. Thallous and mercurous chlorides are thus removed by volatilization. The cold residue is digested with 4 drops of strong ammonia water to dissolve silver chloride, and the contents of the crucible are then evaporated to dryness. Three drops of buffer solution and one drop of sodium rhodizonate are added. If the chloride precipitate contained lead, a red precipitate or coloration will appear. It is necessary to dissolve the silver chloride in ammonia because this salt melts and encloses lead chloride, which may thus be shielded from the sodium rhodizonate.

This procedure, which can also be used to detect lead in the presence of all other metals, is far more sensitive than the usual method of dissolving the lead chloride in hot water and then adding a suitable reagent. The limiting proportion was determined in about 5 mg. of the mixed chlorides; the value is Pb:Ag = 1:5000.

In the presence of barium: The test solution is treated with concentrated sulfuric acid and then taken to definite fuming.

The mixed sulfates are brought onto a filter by means of alcohol and washed with this solvent until the sulfate test is no longer given. About 50 mg. of the washed precipitate are transferred to a spot plate, thoroughly mixed with 5 drops of a saturated solution of sodium acetate in 6 *N* acetic acid, and then dried by a current of heated air. One drop of water and 1 drop of sodium rhodizonate solution are then added. On stirring, the mass becomes violet if lead is present. If only minute quantities of lead are suspected, it is well to run a parallel test with pure barium sulfate. Limiting proportion, Pb:Ba = 1:10,000.

Differentiation of Lead Sulfate and Barium Sulfate

Chemical Basis: Barium sulfate is totally resistant to sodium rhodizonate. This is evidenced by the fact that barium rhodizonate is decomposed by dilute sulfuric acid. In contrast, lead sulfate reacts with this reagent to form a colored lead rhodizonate, that is not attacked by a buffer solution whose pH = 3 (approx.). This difference in behavior can also be used to distinguish lead sulfate from strontium sulfate and calcium sulfate.

Lead sulfate is ordinarily differentiated from the other slightly soluble, white sulfates by the fact that it is soluble in ammonium acetate solution. A complex lead acetate is formed and the solution yields black lead sulfide when treated with hydrogen sulfide. This method is successful only when a fair quantity of lead sulfate is involved, and also presupposes the absence of other metals that form black sulfides.

Traces of lead sulfate can be detected by the method described here. It can also be used to reveal the presence of lead in the company of other metals that cannot be thrown down as sulfates.

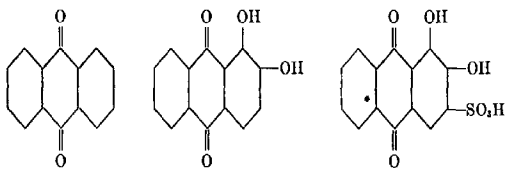
Procedure: Solutions of barium nitrate and lead nitrate, in test tubes, are diluted until they give distinct turbidities on the addition of dilute sulfuric acid. A drop of each of the suspensions is transferred, with the aid of a pipette, to marked places on a filter paper. The drops are allowed to soak in, and the excess acid is then washed away by drops of water. The washed paper is then placed on a spot plate and spotted first with a freshly prepared solution (2 per cent) of sodium rhodizonate, and then with the tartaric acid -bitartrate buffer solution (see p. 134). A red-violet spot or ring appears on the fleck containing lead sulfate. The spot containing barium sulfate becomes discolored after 1 or 2 minutes.

The experiment may be repeated on a spot plate with lead sulfate and barium sulfate powders. It will also succeed with solutions that are so dilute that they give an almost invisible turbidity when treated with dilute sulfuric acid.

As little as 4 γ strontium in the presence of 80 times as much barium can be detected as follows: Filter paper is impregnated with a saturated solution of potassium chromate, and dried. A drop of the neutral test solution is placed on the paper. Insoluble barium chromate deposits, whereas the strontium salt does not react. After about one minute, the spot is treated with the sodium rhodizonate solution and a brown red fleck or ring is formed if strontium is present.

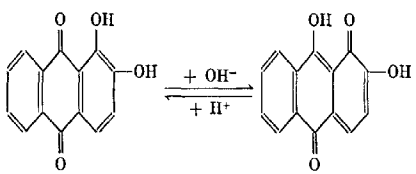
7. ALIZARIN AND OTHER HYDROXY ANTHRAQUINONES

The replacement of hydrogen in the two benzene rings of anthraquinone (I) leads to numerous hydroxy compounds which are often used as mordant dyes. Polyhydroxy anthraquinones having an —OH group adjacent to one of the two —CO groups are of interest as regards spot testing. Examples are alizarin (II), which is slightly soluble in water, and alizarin sulfonic acid or its sodium salt (III), which are quite soluble in water.



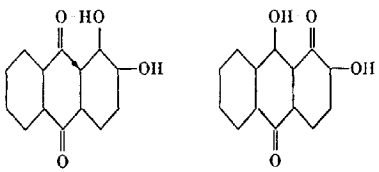
(I) Anthraquinone (II) Alizarin = 1,2-dihydroxyanthraquinone (III) Alizarin sulfonic acid

Alcoholic and aqueous solutions of alizarin and alizarin sulfonic acid are yellow. On addition of ammonia or alkali the solutions turn violet; the yellow color is restored on acidification. This reversible color change permits the use of alizarin as an acid-base indicator for the pH range 5.5 to 6.8. When color changes accompany the formation of salts of acid or basic organic compounds it is always an indication that the salts have a structure differing from that of the parent acid or base. In the case of alizarin the following change is possible when the salt is formed:



The structural formulas of the two tautomeric acid and salt forms show that in the salt a continuous band of conjugated double bonds runs through the *whole* three-ring system, whereas in the acid form only the two lateral benzene rings possess conjugated double bonds. It is well known that such accumulations of conjugated double bonds have a chromotropic effect: i.e., the color is deepened. Therefore, it can be assumed that the yellow color of alizarin (or alizarin sulfonic acid) in acid solution is due to the quinone nucleus. On the other hand, the violet color of the soluble alkali salts results from the development of the long chain of conjugated double bonds with retention of a para-quinoid position of two —CO groups. The same changes in constitution are also possible, with other polyhydroxy anthraquinones.

The acid as well as the salt form of alizarin possesses one —OH group in such close proximity to one —CO group that an auxiliary valence linkage of the H atom of the hydroxyl group to the O atom of the —CO group is possible:



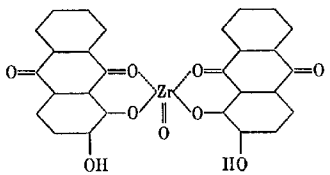
In the light of the foregoing coordination formulas, alizarin itself can be regarded as an inner complex compound of hydrogen. This view, which leads to the conclusion that the two —OH groups, because of auxiliary valence binding, are not equivalent, is supported by the fact that only mono-alkali salts, namely alkali salts of the —OH group in the 2-position, are easily available, and not the di-alkali salts, as might be expected from the phenolic nature of the two —OH groups.

Inner complex compounds of metals and non-metallic groups are often colored because of rings produced by reason of principal and auxiliary valence activity. This is particularly true if one (or both) of the salt-forming components is colored. The spatial relationship of —CO and —OH groups in the alizarin offer possibilities of producing inner complex compounds in both the acid and salt forms. Consequently, it may be expected that alizarin can function as a color reagent in acid as well as in alkaline solution. Such color reactions are known; in fact, they are the basis of the tests for aluminum, zirconium, and boric acid.

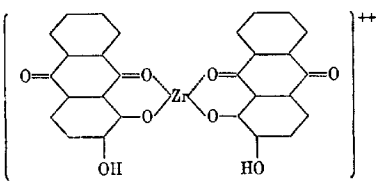
Detection of Zirconium

Chemical Basis: Formation of acid-stable red zirconium alizarinate. Alizarin produces colored precipitates with Al, Be, Ti, and Th salts only in the presence of alkali. Hence, the preliminary, or at least the simultaneous, formation of the metal hydroxide is necessary for production of the lake. In contrast, zirconium ion is the only metal ion which reacts with alizarin in acid as well as alkaline media. A red-violet precipitate forms with alkaline alizarin while in acid solution, depending on the concentration of Zr^{++++} and H^+ ions, a redder precipitate or coloration results.

It is probable that the color reaction in acid solution involves the zirconium not as Zr^{++++} ion but as ZrO^{++} (zirconyl) ion. Accordingly, an inner complex salt of the aci-form of alizarin (see p. 136) is formed and has the coordination formula (I). It is entirely possible, however, that a colored inner complex cation (II) is produced. It has not yet been determined with certainty which of the two formulas is correct.



(I)



(II)

Alizarin sulfonic acid and other polyhydroxy anthraquinones, having at least one —OH group adjacent to a —CO group, act like alizarin.

Procedure: The test solution, as nearly neutral as possible, is treated in a micro-crucible with a drop of an alcoholic solution of alizarin. Red to violet colors are produced by alizarin and other lake formers. If a drop of 1 *N* hydrochloric acid is added, only the zirconium compound will remain. An intense red-violet color and precipitate indicates the presence of large amounts of zirconium; small quantities give a pink coloration.

Identification Limit: 0.5 γ zirconium.

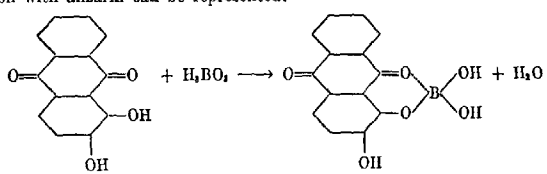
Concentration Limit: 1:100,000.

The reagent is prepared by treating alizarin solution drop-wise with dilute hydrochloric acid until the color becomes pure yellow. An equal volume of alcohol is then added and the solution filtered.

Application in the presence of other materials: The foregoing procedure permits the detection of 1.6 γ zirconium in the presence of 500 times as much aluminum, and of 1 γ zirconium in the presence of 500 times as much thorium. Interference with the clear discernment of small quantities of the red zirconium-alizarin compound need be feared only when considerable concentrations of colored ions are present. On the other hand, the alizarin reaction can be hindered by anions which form stable complex ions with zirconium ions. Examples are fluoride, oxalate, and large quantities of sulfate.

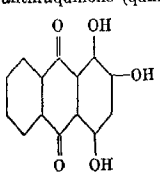
Detection of Boric Acid

Chemical Basis: Formation of the inner-complex boric acid ester of hydroxy anthraquinones. All polyhydroxy anthraquinones are phenolic in nature and consequently, like tertiary alcohols, form esters with acids. Polyhydroxy anthraquinones are soluble in concentrated sulfuric acid; the esterification can occur in this solution where the solvent also functions as the dehydrating agent. If boric acid is warmed with sulfuric acid solutions of hydroxy anthraquinones, inner-complex boric esters are formed. A characteristic color change accompanies the reaction. For instance, the reaction with alizarin can be represented:

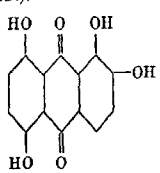


The boric acid is written here in the ortho-form; an analogous equation is valid for meta-boric acid, HBO_2.

Boric acid can be detected not only with alizarin (or alizarin sulfonic acid) but also with 1,2,4-trihydroxy anthraquinone (purpurin) and 1,2,5,8-tetrahydroxy anthraquinone (quinalizarin).



Purpurin



Quinalizarin

Spot reactions intended to detect boric acid by the formation of colored inner-complex esters must be carried out on the residue obtained from evaporation of the test solution, as esterification proceeds rapidly enough only in the absence of water which saponifies the ester into its components. Alkali must be added before evaporating the test solution; otherwise, part of the boric acid will volatilize with the steam.

Procedure: A drop of the weakly alkaline test solution is evaporated to dryness in a microcrucible. Two or three drops of the reagent solution are added and the mixture warmed slightly. If boric acid is present, the following color changes will be observed:

Alizarin sulfonic acid: red to yellow-red;	*identification limit:* 1.0 γ boron.
Purpurin: orange to wine-red;	*identification limit:* 0.6 γ boron.
Quinalizarin: violet to blue;	*identification limit:* 0.06 γ boron.

The reagents are solutions in concentrated sulfuric acid of: alizarin sulfonic acid (0.2 per cent); purpurin (0.05 per cent); quinalizarin (0.01 per cent).

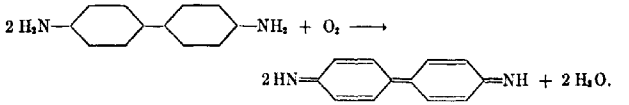
Application in the presence of other materials: Nitrates, chlorates, ferricyanides, and other oxidizing materials interfere with the positive recognition of the colored boric acid esters of polyhydroxy anthraquinones; they produce color changes by reacting with the reagent solutions. Fluorides interfere because of the formation of boron fluoride or fluoboric acid which are incapable of forming esters. Special measures must be taken if considerable quantities of such interfering materials are present. Nitrates and other oxidizing agents can be rendered harmless (reduced) by evaporating the solution with solid hydrazine sulfate. Fluorides are removed by evaporation with precipitated silica and concentrated sulfuric acid (formation of gaseous SiF_4). These operations can be carried out with one or two drops of the test solution in the same crucible in which the subsequent esterification is to be accomplished. The treatment required to remove interfering materials reduces the sensitivity of the boric acid test by about one half.

8. BENZIDINE

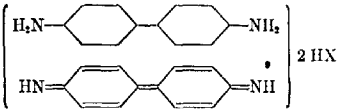
Benzidine, (*p,p'*-diaminodiphenyl, $H_2N-C_6H_4-C_6H_4-NH_2$) is a weak base and is practically insoluble in water. Like many other polyatomic organic bases, it forms salts (acetate, chloride, etc.) which are usually soluble in water. The sulfate is an exception; it is less soluble than barium sulfate. Although water solutions of the acetate or hydrochloride are usually used for analytical purposes, an alcoholic solution of the free base is sometimes used.

Benzidine and its salts are stable in the solid state. The solutions gradually become yellow to brown if they are exposed to light and air. They should, therefore, be kept in brown bottles with tightly fitting stoppers.

Oxidizing agents cannot convert benzidine to a quinone imide:



One molecule of the imide combines with one molecule of benzidine and two equivalents of acid to produce the "meriquinoid" compounds:



If formed from water-soluble benzidine salts, the meriquinoid compounds, are likewise soluble in water and give deep blue solutions containing "benzidine blue."

Even traces of oxidizing agents suffice to produce benzidine blue from benzidine. This reaction is not used in spot test analysis for direct detection of oxidizing agents, but only for detecting higher oxides (NiO_2, PbO_2, etc.) for recognition of auto-oxidation processes [$Mn(OH)_2 \rightarrow MnO_2$], and for detecting materials which, through complex binding, facilitate oxidations (P_2O_5, SiO_2, CN).

Detection of Manganese

Chemical Basis: Formation of benzidine blue through auto-oxidation of manganous hydroxide. Manganous hydroxide produced as shown in (I) undergoes auto-oxidation, that is, it is converted to hydrated manganese dioxide by the oxygen of the air (II):

$$Mn^{++} + 2\ OH^- = Mn(OH)_2 \qquad (I)$$
$$Mn(OH)_2 + O = MnO(OH)_2 \qquad (II)$$

Benzidine can be changed into benzidine blue by the manganese dioxide thus formed, and also by an auto-oxidation process in which atmospheric oxygen functions in the atomic state. It should be noted that the procedure for carrying out this test as a spot reaction on paper exhibits a high sensitivity. The paper, or its surface, plays the rôle of a reactant, probably because of adsorption of the benzidine blue.

Procedure: The test solution should not be too acid. A drop is placed on filter paper, allowed to soak in, and then spotted with a drop of 0.05 *N* sodium or potassium hydroxide. If large quantities of manganese are present, the spot gradually turns brown (formation of MnO_2). Minimal quantities of manganese produce no discernible brown coloration. After the manganese dioxide has formed, either perceptibly or invisibly, the spot

is treated with a drop of benzidine solution. A more or less intense blue fleck appears, depending on the quantity of manganese involved. The blue is not permanent. After some time it disappears or a yellow brown takes its place. The application of a new drop of benzidine solution will regenerate the blue.

Identification Limit: 0.15 γ manganese.

Concentration Limit: 1:330,000.

The reagent is prepared by dissolving 0.05 g. benzidine (base or hydrochloride) in 10 ml. of acetic acid. The solution is made up to 100 ml. with water and then filtered.

Application in the presence of other materials: The benzidine reaction cannot be applied without modification in the presence of oxidizing agents, or of auto-oxidizable materials which, under the conditions of the experiment, likewise cause benzidine to produce a blue coloration. Examples are chromates, ferrocyanides, and salts of cobalt, silver, and cerium. Metal ions which produce colored hydrated oxides, such as $Fe(OH)_3$ and $Cu(OH)_2$, interfere with detection of small quantities of manganese. These obscure the blue and special procedures are required in these cases.

Detection of Manganese in the Presence of Iron, Cobalt, or Cerium

Hydrated ferric oxide has no effect on benzidine but it greatly lowers the sensitivity of the manganese test. For instance, 0.15 γ manganese can be detected in solutions containing manganese alone, whereas 2.5 γ must be present in one drop if 250 γ iron are also present. However, the addition of Rochelle salt will prevent the precipitation of iron. This preventive can be applied by direct spotting of a drop of the test solution on filter paper. Manganese can then be detected by means of the benzidine reaction, even though considerable quantities of iron are present.

Identification Limit: 1 γ manganese
Concentration Limit: 1:50,000
} in the presence of 1000 times as much iron.

Cobaltous hydroxide is auto-oxidizable under the conditions of this test. Accordingly, if manganese is to be detected in the presence of cobalt it is well to convert the cobalt into the soluble, extremely stable $K_3Co(CN)_6$ by adding potassium cyanide and warming. Since, however, excess potassium cyanide interferes with the manganese reaction, through production of complex manganese compounds, the excess is removed by adding several drops of concentrated hydrochloric acid (hood!). This also decomposes the manganese cyanide, while the complex cobalt ion remains unaltered. The steps necessary to mask the cobalt can be carried out with one drop of the test solution in a microcentrifuge tube. It should be noted that the potassium cyanide solution of the complex cobalt salt in contact with benzidine gradually develops a red-violet color.

Identification Limit: 0.5 γ manganese } in the presence of 1200 times as
Concentration Limit: 1:100,000 } much cobalt.

Cerium hydroxide, $Ce(OH)_3$, is likewise auto-oxidizable. If manganese is to be detected in the presence of cerium it is well to warm the test solution for two or three minutes with a little calcium fluoride. This precipitates insoluble cerium fluoride which, on filtering, remains behind with any unchanged calcium fluoride. The manganese test just described is then made with a small portion of the filtrate. The calcium fluoride must be freshly prepared by treating a calcium solution with an alkali fluoride.

Identification Limit: 5 γ manganese } in the presence of 1,000 times as
Concentration Limit: 1:10,000 } much cerium.

Detection of Manganese in the Presence of Copper

Cupric salts form copper hydroxide or basic copper acetate under the conditions of this test. They may thus obscure the production of benzidine blue. Consequently, the detection of small quantities of manganese in the presence of much copper is best accomplished by the procedure recommended for the masking of cobalt. The copper salts are converted into $K_3Cu(CN)_4$ or $Cu_2(CN)_2$.

Identification Limit: 1.6 γ manganese } in the presence of 1500 times
Concentration Limit: 1:30,000 } as much copper.

Detection of Manganese in the Presence of Silver and Thallium

Silver and thallium are best removed from the test solution by adding sodium chloride. The suspension of silver (or thallous) chloride is centrifuged or filtered, and the test for manganese made on the clear solution. If thallic compounds are present, they can be readily reduced to thallous salts with sulfur dioxide, and these then produce slightly soluble TlCl with sodium chloride. The silver and thallium must be removed before testing for manganese, because Ag_2O [or $Tl(OH)_3$] will be formed during the test. Both of these are oxidizing agents. Furthermore, TlOH is auto-oxidizable. Consequently all three will react to produce benzidine blue.

The silver or thallium should not be precipitated directly on the paper by spotting with dilute sodium chloride, because AgCl and TlCl slowly react with benzidine. The blue is due to the photochemical dissociation of these chlorides. Only a little chlorine is set free and slowly, but it suffices to oxidize benzidine.

Detection of Lead

Chemical Basis: Formation of lead dioxide and its reaction on benzidine. Lead dioxide, like manganese dioxide, oxidizes benzidine to benzidine blue. Although MnO_2 is formed by auto-oxidation of $Mn(OH)_2$, lead hydroxide,

in contrast, forms PbO_2 only after action with suitable oxidizing agents. Or lead salts must be treated with oxidizing agents active in an alkaline medium:

$$Pb^{++} + 2\,OH^- + O \rightarrow PbO_2 + H_2O.$$

After the lead dioxide has been formed it is imperative to remove the excess oxidizing agent completely. Even traces of the latter cause benzidine to become blue and can thus lead to false conclusions. Alkali hypobromite is an excellent oxidizing agent for this purpose; it acts quickly and can be removed perfectly. Its oxidizing action and its instantaneous decomposition by ammonia are expressed by the following equations:

$$NaBrO \rightarrow NaBr + O.$$
$$3\,NaBrO + 2\,NH_3 \rightarrow 3\,NaBr + N_2 + 3\,H_2O.$$

The formation of PbO_2 by alkali hypobromite solution and the decomposition of the unused oxidizing agent can be accomplished as spot reactions.

Procedure: A drop of the neutral or acid test solution is placed on filter paper and treated successively with one drop of 3 *N* sodium hydroxide and one drop of bromine water. One or two minutes later the spot is treated with two drops of concentrated ammonium hydroxide. The spot is then touched with an acetic acid solution of benzidine. A more or less intense blue fleck appears, according to the quantity of lead involved.

Identification Limit: 1 γ lead.

Concentration Limit: 1:50,000.

Far smaller amounts of lead can be detected by the following methods: (I). Ten ml. of the test solution, three ml. of 3 *N* alkali and two ml. of bromine water are heated together and the precipitate collected on a retentive paper. The deposit is washed with hot ammonia, followed by hot water, and then spot tested with a solution of benzidine in acetic acid. The identification limit is 10 γ lead in 10 ml.

(II). The procedure is that given in (I), but after warming with the alkali and bromine water, the solution, together with the insignificant precipitate, is transferred to a microcentrifuge tube. A small wad of cotton is placed beforehand in the bottom of the tube. After centrifuging and pouring off the centrifugate, the lead dioxide will be found on the cotton, where it can be purified by centrifuging first with ammonia and then with water. The cotton is then transferred to a white filter paper by means of a platinum wire, and spot tested with benzidine solution. This procedure permits the detection of as little as 4 γ lead in 10 ml.

Application in the presence of other materials: Metal ions which produce higher oxides under the foregoing conditions, or which react directly with

benzidine, will interfere with the test as just described. Such interference is exhibited by Ce^{+++}, Mn^{++}, Co^{++}, Ni^{++}, Bi^{+++}, Tl^{+}, Ag^{+}, Au^{+}. Colored precipitates, which make it difficult to perceive slight blue colorations, lower the sensitivity. In such cases, it is best to prepare an alkaline extract (Plumbite solution), which can contain only TlOH, while the other metals remain behind as insoluble hydroxides. If bismuth is the only material that needs to be considered in the test for lead, it is sufficient to warm the test solution with sodium hydroxide before adding the bromine. Bismuthyl hydroxide, BiOOH, will be formed, and, in contrast to $Pb(OH)_2$ or Na_2PbO_2, it is not converted into a higher oxide by hypobromite. Accordingly, the lead dioxide-benzidine reaction then leads to clear cut conclusions.

Detection of Cyanide

Chemical Basis: Oxidation of benzidine by cupric salts in the presence of cyanide. Cupric salts are weak oxidizing agents, their oxidation potential is too low for them to react directly upon benzidine. Every oxidation (reduction) potential is determined by the equilibrium between the concentrations of the oxidizing and reducting form. In the case of copper, the pertinent equilibrium is

$$[Cu^{++}] \rightleftharpoons [Cu^{+}].$$

It follows that any removal of the Cu^{+} disturbs the equilibrium and therefore brings about a rise of the oxidation potential. Benzidine will be oxidized by Cu^{++} ions, provided halide ions are present to react with Cu^{+} ions to form slightly soluble or non-dissociated cuprous salts. Such halide ions include I^{-}, Br^{-}, CNS^{-}, CN^{-}. This effect can be employed to detect Cu^{++} and also CN^{-}.

The following partial reactions occur when a cupric salt reacts with cyanide:

$$Cu^{++} + 2\ CN^{-} = Cu(CN)_2 \quad (1)$$

$$2\ Cu(CN)_2 = Cu_2(CN)_2 + (CN)_2 \quad (2)$$

$$Cu_2(CN)_2 + 2\ KCN = K_2Cu_2(CN)_4 \quad (3)$$

Equation (3) shows that univalent copper is removed in the form of a soluble complex cyanide. Furthermore, cyanogen is formed (2); it behaves like a free halogen and oxidizes benzidine to benzidine blue.

It is well to make the test by a procedure in which the cyanide is treated with an acid to liberate hydrocyanic acid. This, in turn, is allowed to react with filter paper impregnated with cupric acetate-benzidine acetate solution (Procedure I). Hydrogen cyanide can be evolved from insoluble cyanides by gently warming them with zinc and hydrochloric acid (Procedure II).

Procedure I: A drop of the test solution in a porcelain microcrucible is treated with a drop of dilute sulfuric acid. The crucible is then covered with a strip of filter paper moistened with a drop of reagent solution. A small watch glass placed on the filter paper makes a sufficiently tight seal. A more or less intense blue ring will appear on the white paper, according to the quantity of hydrocyanic acid liberated.

Identification Limit: 0.25 γ cyanogen.

Concentration Limit: 1:200,000.

Procedure II. A drop of the test solution (or several milligrams of the solid specimen) is placed in the decomposition vessel of the apparatus shown on p. 52, Fig. 28. One or two particles of zinc and two or three drops of dilute sulfuric acid are introduced, and the apparatus is closed with the funnel stopper. A strip of filter paper moistened with the reagent solution is laid across the funnel. The apparatus is warmed slightly to liberate the hydrocyanic acid. It is carried along by the hydrogen and causes the paper to turn blue.

Identification Limit: 1 γ cyanogen.

Concentration Limit: 1:50,000.

The copper acetate-benzidine acetate solution is best prepared fresh at the time of the trial; a mixture of the acetates cannot be kept more than two weeks at most. Solution (I) contains 2.86 g. cupric acetate per liter. Solution (II) consists of 475 ml. of saturated (room temperature) benzidine acetate solution plus 525 ml. water. The solutions are stored separately in well-stoppered brown bottles. The reagent is prepared, when needed, by mixing equal volumes of (I) and (II).

Application in the presence of other materials: The test is specific for hydrocyanic acid when volatile oxidizing or reducing compounds are absent. When sulfides are present, the evolution of hydrogen sulfide can be prevented by adding a lead salt which forms acid-stable lead sulfide.

Detection of Phosphoric Acid

Chemical Basis: Oxidation of benzidine by complexly bound molybdenum. Molybdic acid and normal molybdates are weak oxidizing agents and do not affect benzidine. If, however, complex phosphomolybdates are produced, such as $H_3PO_4 \cdot 12\ MoO_3$ or its alkali salts, the complexly bound molybdenum becomes capable of oxidizing numerous materials, including benzidine. This can be shown by comparing the action of a weakly acidified molybdate solution, with and without the addition of phosphate, toward solutions of potassium iodide, a ferrous salt, sulfurous acid, and aniline. The increase in the oxidizing action of the molybdate when phosphate is present is shown by the more rapid bluing of the solution, whose color also continues to deepen.

Under suitable conditions, the formation of benzidine blue as the oxidation product of benzidine is accompanied by the production of lower (blue) oxides of molybdenum ("molybdenum blue") as reduction products of the molybdenum. This simultaneous formation of two blue materials makes it possible to detect traces of phosphomolybdate which cannot be seen with the naked eye or even under a magnifying glass. This also applies to ammonium phosphomolybdate, the familiar egg-yellow crystalline compound which is so often used for the detection and determination of phosphate ion. Accordingly, ammonium phosphomolybdate is produced in this test and then identified by the sensitive benzidine blue reaction.

Procedure: A drop of ammonium molybdate solution in nitric acid is placed on ash-free filter paper and dried in an oven. One drop each of the test solution, benzidine-acetate solution, and finally saturated sodium acetate solution, are added in succession. A blue fleck or ring will appear, according to the quantity of phosphate present.

Identification Limit: 0.05 γ P_2O_5.

Concentration Limit: 1:1,000,000.

It is also permissible to place a drop of the test solution on ash-free filter paper and then spot it successively with one drop each of ammonium molybdate and of benzidine solution. If the paper is then held above an open ammonia bottle, a blue fleck, whose intensity depends on the quantity of phosphate present, will appear when the free mineral acid has been nearly neutralized. The *identification limit* is 1.25 γ P_2O_5 if this procedure is used.

The ammonium molybdate solution is prepared by dissolving 5 g. $(NH_4)_2MoO_4$ in 100 ml. of cold water, and then pouring the solution into 35 ml. of nitric acid (sp. gr. 1.2). The benzidine reagent contains 0.05 g. of the base or hydrochloride dissolved in 10 ml. of glacial acetic acid and then diluted to 100 ml. with water.

Application in the presence of other materials: The yellow precipitate, formed by adding ammonium molybdate to a phosphate in acid solution, is the ammonium salt of the heteropoly acid $H_7P(Mo_2O_7)_6$. Analogous heteropoly acids, which also react with benzidine, because of analogous binding of MoO_3, are formed with arsenic acid, $H_7As(Mo_2O_7)_6$ and silicic acid, $H_8Si(Mo_2O_7)_6$. Under the conditions just described (that is, in the cold), the yellow ammonium arsenomolybdate only deposits with extreme slowness, whereas the phosphomolybdate is immediately precipitated in quantities sufficient to react with benzidine. Consequently, it is possible to detect phosphate in drops that contain 1.5 γ P_2O_5 along with 1.5 mg. As_2O_5. This corresponds to the limiting proportion 1:1000. If traces of P_2O_5 are to be detected in the presence of much As_2O_5, it is best to warm the test solution beforehand with sulfurous acid and then boil out the sulfur

dioxide. The arsenic is thus reduced to As_2O_3, which does not form a precipitate with molybdate.

Soluble silicates react with acid molybdate solution to form yellow soluble silicomolybdic acid. Consequently, it is impossible to use the benzidine test directly for detecting phosphate in the presence of silicates. However, the formation of silicomolybdic acid can be prevented by adding tartaric acid. The precipitation of phosphomolybdate is somewhat delayed under these circumstances but it is nevertheless possible to detect 1.5 γ P_2O_5 in drops which also contain five hundred times this quantity of SiO_2. The test is carried out on filter paper. One drop of the test solution is treated with one drop of tartrate-molybdate solution; held over a heated wire gauze for one minute, spotted with benzidine solution and then developed over ammonia. (The tartrate-molybdate solution contains 15 g. tartaric acid dissolved in 100 ml. of the ammonium molybdate solution described in the preceding section.)

The masking effect of the tartaric acid is due to production of a complex molybdenum tartaric acid. This removes so much of the molybdate ion, which is essential to the formation of the heteropoly acids, that silicic acid and arsenic acid no longer react, and phosphoric acid only reacts slowly (hence the warming). Therefore, when testing for phosphate, the analyst should make certain of the absence of such materials as form stable complex molybdate compounds. Examples are oxalates and fluorides. Considerable quantities of hydrogen peroxide interfere with this test. Permolybdic acid is formed, hindering the precipitation of the phosphomolybdate which is essential to the benzidine reaction.

C. Spot Tests with the Aid of Masking and Demasking Reactions

The technic of spot analysis often employs a device to avoid the disturbing reactions of other similar materials which may be present in the sample. This stratagem is known as the masking of reactions. It involves decrease in the concentration of an ionic or molecular species to the point where the solution no longer exhibits the reactions characteristic of the particular ionic or molecular species. This is accomplished by incorporating the disturbing ion (molecule) into soluble compounds (complexes) which furnish new types of complex ions. The selectivity of a reagent or of a reaction can often be increased by this formation of complexes. The compound whose addition accomplishes the formation of the complex is called the masking agent. Suitable masking agents can not only hinder reactions, but they can also cause the disappearance of reaction products already formed. If this effect can be detected by a discharge or change of color, even when small quantities are involved, the phenomenon can be

applied in spot analysis. The masking of reactions should not be approached by haphazard trial; a working knowledge of the types of complex chemical compounds makes it possible to choose masking agents and masking reactions intelligently and apply them judiciously.

A precipitation or a color reaction which normally proceeds smoothly can be hindered by the presence or addition of a masking agent. Consequently, a masked system represents a retarded reaction which can be accelerated again if the masking agent is removed. This type of demasking is sometimes of great advantage in spot testing, particularly in the detection of a masking or demasking agent.

1. Detection of Cobalt in the Presence of Large Amounts of Iron

Chemical Basis: Formation of colbalt thiocyanate and masking of the iron. Acid solutions of cobalt salts produce an intense blue color when treated with an excess of alkali thiocyanate in the presence of alcohols, aldehydes, or ketones. This effect is due to the formation of a solvate of $Co(CNS)_2$ or $K_2Co(CNS)_4$ with the respective organic solvent. Therefore, if solid alkali thiocyanate is added to a dilute aqueous solution of a cobalt salt and the solution then extracted with ether + acetone or ether + amyl alcohol, the ether layer will be colored deep blue. The thiocyanate reaction for cobalt is very sensitive, but is impaired by the presence of a ferric salt which, with alkali thiocyanate, forms $Fe(CNS)_3$ or $K_3Fe(CNS)_6$ or $Fe(CNS)_2^+$. This red material, like cobalt thiocyanate, is soluble in organic liquids and hence the ferric thiocyanate can completely conceal the blue color due to cobalt thiocyanate. Consequently, small quantities of cobalt will not be detected by this test if iron is present.

The red ferric thiocyanate can be removed completely if an excess of alkali fluoride is added to the aqueous solution or to an ether solution obtained by extraction. Fluorides react with ferric iron to form colorless ferric hexafluoride ion:

$$Fe^{+++} + 6\ F^- \rightleftharpoons FeF_6^{---} \qquad (1)$$

Ferric thiocyanate dissociates, though only to a slight extent:

$$Fe(CNS)_3 \rightleftharpoons Fe^{+++} + 3\ CNS^- \qquad (2)$$

Consequently, the addition of excess fluoride to a solution of $Fe(CNS)_3$ will remove Fe^{+++} ions (1) with the result that the supply of these ions is replenished (2) by the $Fe(CNS)_3$ (or the ferric complexes), until this compound has been exhausted and its interfering color thus discharged.

Cobalt thiocyanate is absolutely stable toward fluoride because no complex cobalt fluoride compound is formed. Consequently, if the iron is masked by means of fluoride, cobalt can be identified with certainty in the presence of considerable quantities of iron.

Procedure: A drop of the acidified test solution is treated on a spot plate with five drops of a saturated acetone solution of ammonium thiocyanate. A green to blue color appears, depending on the quantity of cobalt present. If the blue color is indistinct or concealed by a red color, a few milligrams of ammonium fluoride is added. If cobalt is present a blue or green will remain after the red of the ferric thiocyanate has disappeared. In doubtful cases, it is best to repeat the experiment with one or two drops of the test solution, but the ammonium fluoride should be added *before* the ammonium thiocyanate.

Identification Limit: 0.5 γ cobalt.

Concentration Limit: 1:100,000.

Application in the presence of other materials: The masking of the ferric thiocyanate formation permits the identification of as little as 1 γ cobalt in the presence of 1,000 times this quantity of iron in three drops of a cobalt solution. Nickel salts interfere only when present in considerable quantities; they then produce a light blue color. Consequently, the test solution should not contain more than 2 per cent of nickel.

2. Detection of Palladium

Chemical Basis: Reaction with mercuric cyanide in the presence of diphenylcarbazide. Mercuric cyanide is one of the few soluble binary salts whose ionic dissociation is quite low. Consequently, its aqueous solutions are practically non-conductors and they do not exhibit the precipitation and color reactions characteristic of Hg^{++} and CN^- ions. Mercuric ions can be masked by the addition of cyanide and *vice versa*. Because of the slight dissociation, solutions of this salt do not respond to the sensitive color reaction (violet to blue precipitate) for Hg^{++} ions with diphenylcarbazide. Likewise, silver salts produce no precipitate when added to mercuric cyanide because the concentration of cyanide ions is not sufficient to exceed the solubility product of silver cyanide. Only palladium ion reacts with mercuric cyanide to form white, slightly soluble palladium cyanide with liberation of Hg^{++} ions:

$$Hg(CN)_2 + Pd^{++} \rightarrow Pd(CN)_2 + Hg^{++}.$$

If this precipitation is made in a colorless solution of mercuric cyanide which also contains diphenylcarbazide, even minute quantities of palladium can be detected by this demasking of Hg^{++} ions and the subsequent color reaction with diphenylcarbazide.

Procedure: One drop of a 5 per cent solution of mercuric cyanide containing a few drops of an alcoholic solution of diphenylcarbazide is treated with one drop of the acidified test solution, and warmed gently. A violet coloration appears if palladium is present. The test is best made in a microcrucible.

Application in the presence of other materials: The foregoing is a sure test for palladium, but chromates and molybdates must be absent as they also produce a violet coloration with diphenylcarbazide. Chromate can be reduced with sulfur dioxide and the molybdate-diphenylcarbazide reaction maybe prevented by masking the molybdate with oxalic acid (cf. p. 148). Considerable quantities of colored ions naturally make the detection of the violet coloration difficult, especially when only small amounts of palladium are present. In such cases it is easier to determine the appropriate shades by comparing the color with that given by a blank.

3. Detection of Silver Halide

Chemical Basis: Decomposition of $K_2Ni(CN)_4$ by silver halide in the presence of dimethylglyoxime. The solution of nickel cyanide in potassium cyanide is light yellow; it contains the complex potassium nickel cyanide, $K_2Ni(CN)_4$. This compound shows all the reactions of soluble cyanides, but its nickel is masked toward dimethylglyoxime because it furnishes practically no Ni^{++} ion since this has become a constituent of a stable complex anion. Consequently, it is possible to prepare solutions of $K_2Ni(CN)_4$ and dimethylglyoxime in which there is no precipitate of red nickel dimethylglyoxime. All silver halides dissolve in excess alkali cyanide to form complex cyanides. If a solution of potassium nickel cyanide is used to dissolve the silver halide the reaction produces nickel cyanide:

$$AgHal + K_2Ni(CN)_4 \rightarrow KAg(CN)_2 + Ni(CN)_2 + KHal.$$

This can then react with dimethylglyoxime and form the characteristic red precipitate. The reaction of the slightly soluble nickel cyanide with dimethylglyoxime is slow but it can be speeded up by ammonia, which dissolves $Ni(CN)_2$. Consequently, a properly prepared solution containing $K_2Ni(CN)_4$, ammonia, and dimethylglyoxime can be employed as a quick acting reagent which will react with all silver halides, either when freshly precipitated or after aging for weeks.

Freshly precipitated and well washed $Ni(CN)_2$ is boiled with an insufficient quantity of potassium cyanide solution. The excess nickel cyanide is removed by filtration. The $K_2Ni(CN)_4$ solution prepared in this way is stable. Immediately before the test, several ml. of this solution is treated with several drops of ammonia and several drops of a saturated alcohol solution of dimethylglyoxime. If a precipitate forms the solution should be filtered. The solution containing dimethylglyoxime cannot be kept because the oxygen of the air slowly oxidizes the cyanide and so causes a gradual precipitation of nickel dimethylglyoxime.

Procedure: A few grains of the sample are placed in a depression of a spot plate and treated with one or two drops of the reagent. If a slightly soluble silver salt (AgCl, AgBr, AgCN, AgCNS, AgN_3) is present, either an

intense red coloration or a red precipitate will appear at once. The reaction with silver iodide is somewhat slower, because of its particularly small solubility product but even in this case the formation of red nickel dimethylglyoxime will be seen in about thirty seconds.

Application in the presence of other materials: A demasking of nickel bound into the cyanide complex can be accomplished by all metal ions forming difficultly soluble or undissociated cyanides. Examples are Ag^{+}, Pd^{++} ions, and Hg^{++} ion, which produces soluble undissociated $Hg(CN)_2$. Nickel dimethylglyoxime will also be precipitated under these conditions by organic compounds which consume cyanide, such as the lower aldehydes, particularly formaldehyde.

4. Detection of Fluoride

Chemical Basis: Destruction of zirconium alizarinate. The addition of an acidified bright yellow solution of sodium alizarin sulfonate to hydrochloric acid solutions of zirconium salts produces an intense red violet color. This color reaction, which is characteristic of zirconium, has an identification limit of 0.5 γ zirconium if it is carried out as a spot test. The color is due to the formation of a soluble inner complex zirconium-alizarin compound of unknown constitution. The violet solutions of this complex, like solutions of all complex compounds, represent an equilibrium between the materials which compose the complex:

$$\underset{\text{(red violet)}}{\text{Zr-alizarinate}} \rightleftarrows \underset{\text{(bright yellow)}}{Zr^{++++}} + \text{alizarinate ions.}$$

The equilibrium lies far to the side of the violet zirconium alizarinate, but can be shifted in the other direction, i.e., in favor of the yellow alizarinate ion, if the Zr^{++++} ions are removed from the system. This removal is easily accomplished by the action of fluoride ions which form colorless complex zirconium fluoride ions:

$$Zr^{++++} + 6\ F^{-} \rightarrow ZrF_6^{--}.$$

Consequently, the addition of fluorides to an acidified violet zirconium alizarinate solution is followed by a change to yellow, providing sufficient ZrF_6^{--} ions have been formed to permit perception of the color of the alizarinate ions.

Procedure: One drop of 50 per cent acetic acid and one drop of the neutral test solution are successively placed on zirconium alizarinate paper. A yellow spot is formed on the red violet paper if fluoride is present. If the quantity of fluoride is small it is well to hasten the reaction by warming the paper in a current of steam.

Zirconium alizarinate paper is prepared thus: Dry filter paper is soaked

in a 5 per cent solution of zirconium nitrate and 5 per cent hydrochloric acid, drained, and then placed in a 2 per cent water solution of sodium alizarin sulfonate. The resulting zirconium lake colors the paper red violet.

Identification Limit: 1 γ fluorine.

Concentration Limit: 1:50,000.

Application in the presence of other materials: Anions which combine with zirconium to form complex ions, or to precipitate zirconium, act in the same way as fluorides, namely, by shifting the zirconium alizarinate equilibrium. These include *considerable* quantities of sulfate, which forms complex zirconium sulfuric acid, $H_2Zr(SO_4)_3$; phosphates, arsenates, or oxalates, which produce difficultly soluble normal salts. Oxalates can be removed by a preliminary ingnition of the sample. The removal of sulfates is accomplished by treating the test solution with benzidine hydrochloride which precipitates benzidine sulfate; several drops of the benzidine sulfate suspension are then placed on the reagent paper. The yellow color produced by fluorides can then be easily seen on the other side of the paper.

Since even solid fluorides, such as calcium fluoride, decolorize zirconium alizarinate solution, the following procedure can be used to detect fluoride in the presence of interfering anions. The neutral or alcoholic test solution is treated with calcium chloride, the precipitated calcium salt is collected, ignited and then digested with dilute hydrochloric acid. The calcium fluoride which is insoluble in dilute acids is isolated, transferred to a spot plate, and then treated with a drop of zirconium alizarinate solution containing hydrochloric acid. The latter reagent is a solution of 0.05 g. $Zr(NO_3)_4$ in 50 ml. water and 10 ml. hydrochloric acid, plus a solution of 0.05 g. sodium alizarin sulfonate in 50 ml. water.

5. Detection of Cyanide

Chemical Basis: Decomposition of copper sulfide by formation of complex copper cyanide. If cupric solutions are treated with alkali cyanide they are decolorized immediately. The reaction proceeds in three stages: (1) formation of cupric cyanide; (2) decomposition into cuprous cyanide; (3) solution in the excess cyanide forming colorless complex cyanide. This series of reactions is represented:

$$Cu^{++} + 2\,CN^- \rightarrow Cu(CN)_2 \quad (1)$$

$$2\,Cu(CN)_2 \rightarrow Cu_2(CN)_2 + (CN)_2 \quad (2)$$

$$Cu_2(CN)_2 + 2\,CN^- \rightarrow Cu_2(CN)_4^{--} \quad (3)$$

Despite this complicated course of the reactions, the formation-tendency and stability of the complex cuprous cyanide is so great that hydrogen sulfide or alkali sulfides produce no precipitate when added to solutions containing the $Cu_2(CN)_4^{--}$ ions. Consequently, cyanides mask the precipitation of copper as sulfide. This fact is especially noteworthy as only

a few examples of the masking of sulfide precipitations are known, because the sulfides, in general, have extremely small solubility products. Both freshly precipitated and aged copper sulfide dissolve in alkali cyanide to form $Cu_2(CN)_4^{--}$ ion, and this reaction is the basis of a rather sensitive test for cyanide. The solution of copper sulfide by alkali cyanide can be made in a drop of a copper sulfide suspension, which becomes clear and colorless on the addition of potassium cyanide. It is better to utilize finely divided copper sulfide which has been precipitated in the capillaries of filter paper because, under these circumstances, it is possible to detect even small quantities of cyanide.

Procedure: A drop of the neutral or alkaline test solution is placed on freshly prepared copper sulfide paper. In the presence of cyanide a white ring will be formed.

Copper sulfide paper is prepared thus: Filter paper is impregnated with an ammonical solution of 0.1 g. $CuSO_4 \cdot 5H_2O$ in 100 ml. water, and dried. Immediately before the test the paper is exposed to hydrogen sulfide and thus colored a uniform yellow brown.

Identification Limit: 1.25 γ cyanide.

Concentration Limit: 1:40,000.

Application in the presence of other materials: The foregoing test for cyanide is also valid in the presence of ferrocyanide, ferricyanide, chloride, bromide, iodide, and thiocyanate.

6. Detection of Free Acids or Basic Compounds in Solutions of Aluminum Salts

Chemical Basis: Conversion of Al^{+++} ion into complex $Al(C_2O_4)_3^{---}$ ion. All aqueous solutions of aluminum salts react acid toward acid-base indicators because of the hydrolysis:

$$Al^{+++} + 3\ H_2O \rightleftarrows Al(OH)_3 + 3\ H^+.$$

This hydrolysis is so extensive that even solutions of basic aluminum salts, such as aluminum acetate, $Al(OH)(CH_3 \cdot COO)_2$, react acid despite the fact that they contain the basic OH group.

Addition of neutral alkali oxalate converts the Al^{+++} ions immediately and completely into the complex aluminum oxalate ions:

$$Al^{+++} + 3\ C_2O_4^{--} \rightarrow Al(C_2O_4)_3^{---}.$$

This stable anion does not undergo hydrolysis. Consequently, when the Al^{+++} ions of an aqueous solution of an aluminum salt have been masked by oxalate ions, the solution will exhibit an acid reaction toward indicators only if it originally contained free mineral acid. Solutions of pure aluminum salts become neutral if they are masked by the addition of alkali

oxalate. The action on solutions of basic aluminum salts is analogous; the Al^{+++} ion is masked, but a solution remains which reacts alkaline toward indicators. Consequently, the masking of Al^{+++} ions can be utilized to detect the presence of free acid or basic compounds in aluminum salts.

The following reactions occur

(1) Neutral Al-salts:

$$Al_2(SO_4)_3 + 6\ Na_2C_2O_4 \rightarrow 2\ Na_3Al(C_2O_4)_3 + 3\ Na_2SO_4.$$

(2) Acid Al-salts:

$$H_2SO_4 + Al_2(SO_4)_3 + 6\ Na_2C_2O_4 \rightarrow 2\ Na_3Al(C_2O_4)_3 + 3\ Na_2SO_4 + H_2SO_4.$$

(3) Basic Al-salts:

$$Al(OH)(CH_3{\cdot}COO)_2 + 3\ Na_2C_2O_4 \rightarrow Na_3Al(C_2O_4)_3 + 2\ CH_3COONa + NaOH.$$

Procedure: Several crystals of sodium oxalate are stirred into one or two drops of the test solution on a spot plate. The resulting mixture is tested for free acid or free base with methyl orange or phenolphthalein.

7. Detection of Amorphous Silica

Chemical Basis: Demasking of silver-ammine chromate. Silver chromate (red-brown) dissolves in excess ammonia water and forms silver-ammine chromate. The solution is light yellow because of the presence of CrO_4^{--} ions. Consequently, digestion of freshly precipitated silver chromate with ammonia, followed by filtration, produces solutions in which the precipitation of brown silver chromate is masked because of the presence of ammonia. Such solutions present the equilibrium:

$$(AgNH_3)_2CrO_4 \rightleftarrows 2\ NH_3 + Ag_2CrO_4.$$

This equilibrium of the masked reaction is disturbed by the addition of all materials which consume ammonia; demasking occurs and red-brown silver chromate precipitates. These silver-ammine chromate solutions can thus be used to detect soluble or slightly soluble inorganic or organic compounds which exhibit acid characteristics.

The behavior of the anhydride of silicic acid is particularly interesting and useful. Pure silica in its crystalline form (quartz sand, rock crystal, etc.) does not react with this reagent even though it has been very finely pulverized. In contrast, amorphous silica, either hydrous or ignited, reacts immediately with silver-ammine chromate solutions. Consequently, the demasking of this reagent furnishes a means of distinguishing between crystalline and amorphous silica. The "gangue" remaining after oxide rocks have been dissolved, and the siliceous residues left after the solution of impure metals, alloys, etc. in acids, apparently contain amorphous silica because they react positvely when spot tested with silver-ammine

chromate solution, even after they have been freed of free mineral acid by thorough washing with hot water.

The action of silica on masked silver chromate solution is probably of a different nature than that due to slightly soluble acid compounds. The latter cause the precipitation of silver chromate as the result of a chemical reaction in which the equilibrated ammonia is consumed in the formation of the particular ammonium salt. The direct formation of an ammonium salt is not involved in the case of amorphous silica, particularly that contained in ignited products. It is much more likely that ammonia is rapidly adsorbed on the surface of the finely divided silica; this adsorption likewise disturbs the equilibrium and results in the precipitation of silver chromate.

Procedure: Small quantities of the sample, which must be freed from adhering mineral acids, are treated with two or three drops of silver-ammine chromate solution on a spot plate. A red-brown or brown color immediately develops on the surface of the powder, the intensity depending on the quantity of amorphous silica present.

It should be noted that a drop of the reagent placed on a spot plate gradually precipitates silver chromate on the surface turned toward the air. Loss of ammonia is responsible. This decomposition is slow and the silver chromate formed under these conditions is finely crystalline and floats as a dark brown layer on the surface, whereas the chromate produced by the action of silica adheres to the solid test material.

The behavior of moist and of ignited silica, as well as that of pulverized quartz or sand, toward the reagent solution should be compared with amorphous silica prepared especially for comparison tests. Sodium silicate is evaporated on the water bath with hydrochloric acid, and the residue, amorphous silica, thoroughly washed with hot water. A portion of this comparison test material should be ignited.

Silver-ammine chromate solution is prepared as follows: Silver nitrate solution is treated with potassium chromate. The precipitate is washed with hot water and then shaken with less than the required amount of 6 *N* ammonia water. After standing one hour the suspension is filtered and the filtrate preserved in a tightly stoppered bottle. On long standing the reagent becomes turbid but can be restored by filtration.

Application in the presence of other materials: Acids, acid salts, salts which react acid because of hydrolysis, slightly soluble acid anhydrides such as MoO_3, As_2O_3, As_2O_5, Sb_2O_5, B_2O_3, etc., act toward masked silver chromate solution in the same manner as amorphous silica.

The behavior of tungstic acid is noteworthy. The hydrate, H_2WO_4, reacts immediately, whereas strongly ignited WO_3 is inactive toward silver-ammine chromate solution under the foregoing conditions.

D. Tests by Means of Catalysis Reactions

Catalysts, by definition, are materials which, in minimal quantities, are capable of increasing the rate of chemical reactions. They exert this action only on quite particular reaction systems, without appearing in the stoichiometric equation as a participant in the reaction. Consequently, if a catalyst can be detected by its accelerating action on a characteristic reaction, that is, if the catalyzed reaction can be utilized to detect the catalyst, the demands of greater sensitivity as well as extensive selectivity have been met.

All catalyzed reactions involve an increase in the reaction rate of chemical changes which of themselves proceed slowly. A catalyst cannot change the equilibrium state of a system. Most of the catalyses employed for spot test analysis occur in homogeneous systems. As to the mechanism of this type of acceleration it should be noted that, strictly speaking, a single material does not accomplish the acceleration. A reaction always occurs in which the catalyst is involved as an active participant. The product of this primary reaction contains the catalyst and takes part in a succeeding (secondary) reaction regenerating the catalyst, which then again participates in a new primary reaction, and so on. A cycle of this kind is known as intermediate reaction catalysis. A general representation of this type of catalysis will make these relationships clear. If the reactants of the catalyzed reaction are denoted: A, B, and AB, the catalyst = *cat*, and the labile reaction product of the catalyst = *cat* A, then the partial reactions (1) and (2) can be added to give the net equation (3) of the catalyzed reaction. The catalyst does not appear in this equation. This is in conformity with the foregoing definition of a catalyst.

$$A + cat \rightarrow cat\ A \qquad (1)$$

$$\underline{cat\ A + B \rightarrow AB + cat} \qquad (2)$$

$$A + B \rightarrow AB \qquad (3)$$

Closely related to the true catalyzed reactions are "induced reactions." These are reactions which normally proceed slowly, but whose rate is greatly increased by the simultaneous occurrence of another reaction involving the same reaction partner. The common reactant is called the "actor," its partner in the inducing reaction is the "inductor," and in the induced reaction its partner is the "acceptor." Induced reactions are known which are initiated by such small quantities of inductor that apparently they do not differ from catalyzed reactions. However, the mechanism of these two types of reactions is different in that the inductor of an induced reaction is consumed during the course of the inducing reaction, whereas the catalyst of an intermediate reaction catalysis is constantly regenerated.

1. Detection of Bismuth

Chemical Basis: Catalysis of the $Pb(OH)_2 - SnO_2^{--}$ reaction by bismuth salts. Alkaline solutions of sodium or potassium stannite reduce bismuth salts almost instantly to finely divided metallic bismuth, which then appears as a black precipitate or a black to brown coloration:

$$2\ Bi(OH)_3 + 3\ SnO_2^{--} \rightarrow 2\ Bi + 3\ SnO_3^{--} + 3\ H_2O.$$

At room temperature alkaline stannite reacts very slowly on lead salts. For instance, if a drop of 1 per cent lead nitrate solution is treated on a spot plate with one drop of alkaline stannite solution, the brown color indicating the beginning of the reduction will not appear until 3 to 10 minutes have elapsed. The reaction:

$$Pb(OH)_2 + SnO_2^{--} \rightarrow Pb + SnO_3^{--} + H_2O,$$

which normally proceeds very slowly, is speeded up tremendously if bismuth is precipitated simultaneously by the analogous reaction. This acceleration of the lead reduction is accomplished by quantities of bismuth so small that they themselves cannot be detected by reduction with alkaline stannite solution. Antimony and thallium salts behave in the same manner as lead salts; they are only slowly reduced by stannite, but their reduction occurs rapidly if traces of bismuth salts are present.

Cadmium salts are not reduced at all by stannite, but if minute quantities of bismuth are present the reduction occurs smoothly in warm solutions. This finding is of importance in clearing up the mechanism of the acceleration accomplished by bismuth salts. The reduction of bismuth (III) salts by stannite proceeds through lower labile, extremely reactive oxides of uni- or bivalent bismuth. These, in turn, reduce lead, antimony, thallium, and cadmium salts to the respective metals and the reduced bismuth is reconverted to the trivalent condition.

The catalytic activity of bismuth in the reduction of lead can be used as the basis of a spot test for bismuth under definite prescribed conditions.

Procedure: A drop of the acidified (HCl) test solution, a drop of saturated lead chloride solution, and two drops of stannite solution are placed on a spot plate and stirred together. When considerable quantities of bismuth are present a precipitate of metallic lead and bismuth appears immediately. If smaller quantities of bismuth are involved a distinct brown color appears after 1 to 3 minutes. The color gradually darkens, and finally the lead is completely precipitated. Since lead salts are reduced when alone, though the action is very slow, it is best when testing for small quantities of bismuth to run a blank test with one drop each of hydrochloric acid and lead chloride solution and two drops of stannite.

The stannous chloride solution contains 5 g. $SnCl_2 \cdot 2H_2O$ dissolved in

100 ml. concentrated hydrochloric acid and then dilute to 100 ml. with water. The stannite solution is prepared just before use by mixing equal volumes of 25 per cent sodium hydroxide and stannous solutions. Freshly prepared stannite solutions are required because on long standing alkaline stannite solutions often deposit free tin by the reaction:

$$2\ Na_2SnO_2 + H_2O \rightarrow Na_2SnO_3 + 2\ NaOH + Sn,$$

in which half of the stannite is oxidized and the other half reduced.

Identification Limit: 0.01 γ bismuth.

Concentration Limit: 1:5,000,000.

Application in the presence of other materials: It has been previously mentioned that the reduction of bismuth salts is catalyzed not only by the reduction of lead salts but also by the analogous reaction of antimony and thallium salts. They do not impair this test for bismuth because the reduction of lead and thallium salts by stannite proceeds extremely slowly. The circumstances are different, however, when copper and mercury salts are present because they, especially the latter, are reduced rapidly by stannite. These interferences can be overcome by simple measures. In the presence of copper it suffices to precede the addition of stannite with a drop of 5 per cent potassium cyanide solution. This forms colorless $K_2Cu_2(CN)_4$, which does not affect stannite. In this way, and if a parallel test with 1 per cent copper sulfate solution is made, it is possible to detect 0.1 γ bismuth in the presence of 10,000 times this quantity of copper.

Mercury salts must be removed beforehand. This is conveniently done by evaporating a drop of the test solution in a porcelain microcrucible and igniting the residue. The ignition residue is taken up in one drop of 1 *N* hydrochloric acid and tested for bismuth by the procedure previously outlined. In this way, 0.05 γ bismuth can be detected in the presence of 10,000 times this quantity of mercury.

2. Detection of Silver

Chemical Basis: Catalytic hastening of the reduction of manganese (III) and cerium (IV) salts by hydrochloric acid. The higher oxides of manganese, the salts of manganese (III) and (IV), as well as cerium (IV) salts are reduced with liberation of chlorine if they are warmed with concentrated hydrochloric acid:

$$MnO_2 + 4\ HCl \rightarrow MnCl_2 + 2\ H_2O + Cl_2.$$

$$2\ Ce(NO_3)_4 + 8\ HCl \rightarrow 2\ CeCl_3 + 8\ HNO_3 + Cl_2.$$

No liberation of chlorine, or very little, occurs at room temperature and with dilute hydrochloric acid (up to 2.5 *N*). Consequently, it is possible to prepare brown solutions of $MnCl_3$ or yellow solutions of $CeCl_4$ which

can be kept for some time. On warming, these solutions decompose evolving chlorine and forming colorless solutions of $MnCl_2$ and $CeCl_3$, respectively. However, if these fairly stable colored solutions containing hydrochloric acid are treated with traces of silver salts the color fades almost instantly. The silver ion, or the silver chloride present in hydrochloric acid solution, acts as a positive catalyst in these reactions.

It is probable that the mechanism of the catalyzed production of chlorine involves intermediate formation of $HAgCl_2$, resulting from the union of silver chloride and hydrochloric acid:

$$AgCl + HCl \rightarrow HAgCl_2.$$

Silver chloride dissolves in strong hydrochloric acid, in accordance with this equation, and the hydrochloric acid coordinated on silver chloride is more reactive than free hydrochloric acid. The activation of hydrochloric acid by silver chloride is not limited to Mn (III), Mn (IV) and Ce (IV) salts, but chromates and vanadates may be reduced more quickly by hydrochloric acid if small quantities of silver salts are present.

The catalyzed liberation of chlorine with Mn (III) and Ce (IV) salts is brought about by such minute quantities of silver that this can be used as the basis of a test for both soluble and slightly soluble silver salts.

Procedure: Three drops each of the reagent solution Mn (III) or Ce (IV) and two drops of dilute hydrochloric acid are placed in adjoining depressions of a spot plate. A drop of the test solution is added to one of the mixtures, a drop of water to the other. The color is discharged more or less rapidly according to the quantity of silver present.

The reagents are prepared thus: Mn (III) solution. 0.6 g. $MnSO_4 \cdot 4H_2O$ dissolved in 60 ml. water and 20 ml. concentrated HCl is treated with 10 ml. 0.1 *N* $KMnO_4$ solution and shaken thoroughly. Just before use, 15 ml. of this solution is diluted with 50 ml. HCl (1:3).

Ce (IV) solution. 0.25 g. cerium ammonium nitrate is treated with 10 ml. dilute nitric acid and diluted to 100 ml. with water.

Identification Limit: 0.04 γ silver
Concentration Limit: 1:120,000
} in presence of Mn (III) salt

Identification Limit: 0.05 γ silver
Concentration Limit: 1:1,000,000
} in presence of Ce (IV) salt

Application in the presence of other materials: The foregoing tests are specific for silver, but the presence of considerable quantities of colored ions interfere because it is difficult to see the decolorization of the Mn (III) or Ce (IV) solutions. The catalyzed reaction can also be employed to detect silver in the mixed precipitate ($AgCl + Hg_2Cl_2 + TlCl$) obtained on the addition of hydrochloric acid in the systematic scheme of analysis.

The chloride precipitate is washed, dried, and ignited in a microcrucible to eliminate $HgCl_2$ and TlCl. The residue is cooled and treated with one or two drops of Mn (III) or Ce (IV) solution. If silver is present the color is discharged.

3. Detection of Copper

Chemical Basis: Catalysis of the Fe^{+++}—$S_2O_3^{--}$ reaction by copper salts. The net reaction between ferric salts and alkali thiosulfates can be represented by the equation:

$$2\ Fe^{+++} + 2\ S_2O_3^{-} \rightarrow 2\ Fe^{++} + S_4O_6^{--}.$$

This reaction occurs in two stages. A stable violet complex anion $Fe(S_2O_3)_2^-$ is formed first by the union of one ferric with two thiosulfate ions. This colored material reacts at a measurably slow rate with ferric ion:

$$Fe(S_2O_3)_2^- + Fe^{+++} \rightarrow 2\ Fe^{++} + S_4O_6^{--}.$$

Copper salts accelerate the second partial reaction and so speed up the total reaction between ferric ion and thiosulfate ion. In other words, the red coloration due to the intermediate ferri-thiosulfate disappears far more quickly in the presence of copper ions than in a solution containing no copper. This catalysis probably involves the intermediate formation of cuprous salts produced by reduction of cupric salts with thiosulfate. These react quickly with ferri-thiosulfate regenerating the cupric salt.

If the reaction between ferric salts and thiosulfate occurs in the presence of a thiocyanate, the latter will not only act as indicator of the ferric salt remaining, but will also retard the reaction. Accordingly, a comparison of the times necessary for the decolorization of the ferric solution containing thiocyanate, in the presence and absence of copper, can be used to detect extremely minute quantities of copper.

Procedure: A drop of the test solution and a drop of distilled water are placed next to each other on a spot plate. Each is treated with one drop of ferric thiocyanate solution and three drops of 0.1 *N* sodium thiosulfate. The solutions are then mixed with a glass rod. The copper-free solution will lose its color in about 1.5 to 2 minutes. The test solutions will fade almost immediately if as little as 1 γ copper is present. With still smaller quantities the differences in the times are easily discerned.

The ferric thiocyanate solution contains 1.5 g. $FeCl_3$ and 2 g. KCNS dissolved in 100 ml. water.

Identification Limit: 0.02 γ copper.

Concentration Limit: 1:2,500,000.

Application in the presence of other materials: The reaction rate changes

quite considerably with small variations in the relative concentrations of iron: thiocyanate: thiosulfate. The temperature and the acid content of the test solution also influence the reaction velocity. When traces of copper are suspected it is well to run a blank under the same conditions. The possible presence of copper in the reagents must be guarded against; as well as the possible delivery of traces of copper by the glass. Such extraneous copper can be completely removed by shaking the suspected materials with calcium fluoride or talc and then centrifuging. The necessity of these precautions can be demonstrated by comparing the decolorization times in distilled and purified water, and in water in which a copper wire has been immersed for thirty seconds.

All arsenic compounds retard the acceleration; aluminum, zinc, and nickel salts, to a lesser degree.

4. Detection of Sulfite

Chemical Basis: Induced oxidation of $Ni(OH)_2$ to $Ni(OH)_3$. Certain oxidations, which normally proceed slowly, are accelerated markedly if SO_2 is simultaneously oxidized to SO_3 (or sulfite to sulfate). For instance, the oxidation of As_2O_3 by bromate is quite slow, but becomes practically instantaneous when sulfite is present. The latter is oxidized immediately to sulfate by bromate. Consequently, the SO_3^{--}–BrO_3^- reaction induces the slow AsO_3^{---}–BrO_3^- reaction.

The oxidation of SO_2 by the oxygen of the air (auto-oxidation) likewise induces other oxidations, including the conversion of $Ni(OH)_2$ to $Ni(OH)_3$. This is particularly remarkable because normally the change is accomplished only by strong oxidizing agents such as halogens, persulfate, etc. For instance, if a dish containing sulfurous acid is placed in a desiccator, along with a watch glass which has nickel hydroxide spread on it, the green $Ni(OH)_2$ turns black due to the formation of $Ni(OH)_3$. The mechanism of this induced reaction probably involves the preliminary formation of an addition compound of molecular oxygen (actor) on SO_2 (inductor):

$$SO_2 + O_2 \rightarrow SO_2 \cdot O_2.$$

This then reacts with the $Ni(OH)_2$ (acceptor):

$$2\ Ni(OH)_2 + SO_2 \cdot O_2 + 2\ H_2O \rightarrow H_2SO_4 + 2\ Ni(OH)_3.$$

The oxidation of $Ni(OH)_2$ when stirred with SO_2 and air can be used to detect sulfite, because the color change is quite striking. If the quantity of SO_2 is very small, traces of the $Ni(OH)_3$ formed can be made visible by spotting with benzidine, which produces a blue color (cf. p. 141).

Procedure: A drop of the test solution or several small particles of the solid is placed in the bulb of the apparatus (Fig. 25) described on p. 51.

A little freshly precipitated nickel hydroxide, washed free of alkali, is put on the glass knob extending into the vessel. One or two drops of hydrochloric acid (1:1) are added, the apparatus closed, and the SO_2 liberated by warming gently. The green layer of $Ni(OH)_2$ turns gray to black, according to the quantity of sulfite present. If only minute quantities of sulfite are involved, the nickel hydroxide on the knob should be exposed to the action of the SO_2, and then transferred to a quantitative filter paper. A blue color will develop when the spot is treated with benzidine acetate solution, because colorless benzidine is oxidized to "benzidine blue" by $Ni(OH)_3$.

Alkali-free, washed nickel hydroxide is prepared by treating $NiCl_2$ solution with NaOH. The benzidine acetate solution contains 0.05 g. benzidine base or hydrochloride dissolved in 10 ml. acetic acid, diluted to 100 ml. with water, and then filtered.

Identification Limit: 0.4 γ sulfur dioxide.

Concentration Limit: 1:125,000.

Application in the presence of other materials: The test is specific for sulfite, if thiosulfate and sulfide are absent. The former generates sulfur dioxide when acidified; the latter evolves hydrogen sulfide.

Finely divided sulfur produces sulfur dioxide when insolated. This can be demonstrated as follows: Nickel hydroxide is streaked on filter paper, several particles of sulfur are added, and the specimen exposed to direct sunlight for a few minutes. A distinct blue appears if the material is then spotted with benzidine acetate solution.

5. Detection of Sulfur Combined as Sulfide

Chemical Basis: Catalytic acceleration of the iodine-azide reaction. If solutions of sodium azide and iodine (as iodine-potassium iodide solution) are mixed, no reaction occurs, even after months of standing. However, if the brown iodine-azide solution is treated with a little sodium sulfide or sodium thiosulfate, a vigorous evolution of free nitrogen occurs at once, with accompanying consumption of the free iodine. The reaction:

$$2\ NaN_3 + I_2 \rightarrow 2\ NaI + 3\ N_2,$$

which is thus initiated by sulfide, can also be started by thiosulfate and thiocyanate. The action of the sulfur bearing compounds is not disclosed by this equation, and since even traces of them suffice to accelerate the iodine-azide reaction, this is a typical instance of catalysis.

This effect on the iodine-azide reaction is exerted by all materials, either organic or inorganic, containing sulfur in *sulfidic* linkage. Solubility plays no rôle here; even the least soluble metal sufides (HgS, As_2S_3) act promptly. Consequently, mere traces of materials containing sulfide-bound sulfur can be detected by the evolution of nitrogen from an iodine-azide solution.

The mechanism of the catalytically accelerated or initiated reaction between iodine and azide probably differs when soluble or insoluble sulfides respectively are involved. An intermediate reaction catalysis appears obvious if it is assumed that the oxidation of sulfide by iodine does not lead directly to iodide and free sulfur as shown in (3), but that a labile compound is formed (1) and this rapidly decomposes (2). Summation of (1) and (2) gives the net Equation (3).

$$Na_2S + I_2 \rightarrow NaSI + NaI \quad (1)$$

$$NaSI \rightarrow NaI + S \quad (2)$$

$$Na_2S + I_2 \rightarrow 2\,NaI + S \quad (3)$$

When sodium azide is present it can act directly on NaSI and so hinder the decomposition shown in (2). Consequently, the following series of reactions may occur in the system Na_2S—I_2—NaN_3 in which the sulfide appears only in the intermediate Reactions (4) and (5) but not in (6).

$$Na_2S + I_2 \rightarrow NaSI + NaI \quad (4)$$

$$NaSI + 2\,NaN_3 \rightarrow NaI + Na_2S + 3\,N_2 \quad (5)$$

$$I_2 + 2\,NaN_3 \rightarrow 2\,NaI + 3\,N_2 \quad (6)$$

This mechanism of an intermediate reaction catalysis can also be appropriately applied to thiosulfate and thiocyanate, which likewise contain sulfide-bound sulfur. In the case of thiocyanate the formation of $NaIS_2O_3$ (from $Na_2S_2O_3$ and iodine) can be assumed. This active intermediate compound then reacts with NaN_3.

The assumption of an intermediate reaction catalysis meets with difficulties in the case of slightly soluble metal sulfides, which likewise immediately initiate the iodine-azide reaction. It is hard to justify the formation of SI^- ions in the cases of mercuric sulfide and sulfide ores, whose solubility in water is infinitesimal. It seems more logical to postulate an adsorption of iodine on the surface of the solid sulfides. This adsorption activates the iodine and makes it more reactive toward sodium azide.

The catalytic acceleration of the iodine-azide reaction can be used to detect soluble and insoluble sulfides; also thiosulfates and thiocyanates. As the reagent solution is usually in excess, the formation of bubbles of nitrogen, rather than the decolorization of the iodine, is observed.

Procedure: (a) Soluble sulfidic compounds. A drop of the test solution is mixed with a drop of iodine-azide solution on a watch glass. (A sheet of black glossy paper behind the watch glass improves the visibility.) Immediate liberation of gas bubbles indicates the presence of sulfide, thiosulfate, or thiocyanate. If small quantities only are present, little bubbles adhering to the watch glass will be seen in one to two minutes.

Identification Limits:	*Concentration Limits:*
0.02 γ sodium sulfide	1:2,500,000
0.04 γ sodium thiosulfate	1:1,250,000
1.5 γ potassium thiocyanate	1:33,000

Still smaller quantities of sulfide, thiosulfate, or thiocyanate can be detected if a micro-drop of the solution is evaporated in the loop of a platinum wire. This is accomplished easily if the wire is heated to glowing about 2 centimeters from the loop. The rest of the procedure is that given in (*b*) for the detection of minute quantities.

(*b*) *Solid sulfidic compounds.* Very small quantities of solid sulfides can be detected by covering a particle of the specimen on a watch glass with a drop of iodine-azide solution. After a short time, the bubbles can be seen on the top of the drop.

Much smaller quantities can be detected if a drop of sodium azide-iodine solution is placed in a capillary or in an Emich microcentrifuge tube. A particle of the specimen is then introduced into the suspended drop with the point of a platinum wire bent at about 45° (Fig. 36). If sulfide is present bubbles that can easily be seen with the naked eye or a magnifying glass will rise in the capillary or in the constricted portion of the microcentrifuge tube. Another sensitive method of conducting this test for solid sulfides is described on p. 214.

The iodine-azide solution contains 1 g. sodium azide and 1 g. potassium iodide dissolved in 3 ml. water. A small crystal of iodine is added.

Application in the presence of other materials: The catalyzed iodine-azide reaction is specific for sulfide-bound sulfur in soluble and slightly soluble compounds. Free sulfur, sulfur(IV), sulfur(VI) (sulfite, sulfate) do not react. Selenides and tellurides, which ordinarily resemble sulfides in their reactions, do not respond to this test.

Sulfo salts, such as $2HgS \cdot HgCl_2$ or similar molecular compounds of sulfides, react promptly. They can thus be detected in the presence of sulfates, free sulfur, and so forth. For this reason the iodine-azide reaction is useful for the solution of many special problems (see p. 229).

An interesting application is the detection of thiocyanate in the presence of compounds which do not permit the use of the familiar ferric-thiocyanate reaction. Examples are oxalic acid, tartaric and other organic hydroxy acids, phosphoric acids, ferrocyanides, iodides. In the presence of these organic acids or of phosphoric acid, the formation of complex iron salts prevents the thiocyanate reaction completely or to a great extent; ferrocyanides produce Prussian blue. If iodides are present the thiocyanate test cannot be applied because iodine is liberated:

$$2\ Fe^{+++} + 2\ I \rightarrow 2\ Fe^{++} + I_2,$$

and the color makes it impossible to see the red ferric thiocyanate. These anions, with the exception of phosphate and ferrocyanide, do not interfere with the iodine-azide test for thiocyanate. Phosphate and ferrocyanide decrease the sensitivity of this test for thiocyanate only when they are present in considerable quantities.

E. Spot Tests by Induced Precipitations

Induced precipitation may be defined as the initiation of a precipitation which does not occur by itself or which proceeds at an immeasurably slow rate, through the simultaneous formation of another precipitate by the same precipitant. Accordingly, the formation of the main precipitate and of the coprecipitated material requires a common ion known as the "actor." The other ion involved in the precipitation of the main precipitate is the "inductor," that of the coprecipitated material is the "acceptor." The inducing precipitation must always occur to a considerable extent so that the induced precipitation of small quantities of material will be assured. This requirement and the definite consumption of the inductor distinguishes induced precipitations from catalyzed reactions, in which a primary reaction (of the catalyst) also initiates a second reaction (of the catalyzed system).

The formation of mixed crystals, occlusion, adsorption, and the formation of complex compounds all play a part in induced precipitations. Frequently the single effects cannot be kept apart. Some proceed concurrently, others in turn.

Induced precipitations can be utilized for analytical purposes. The coprecipitation may be revealed by an abnormal color, or by a more copious formation of precipitate. Sometimes the induced precipitation accomplishes a selective accumulation of the acceptor which, after the precipitate has been dissolved, can be identified by an appropriate special reaction.

1. Detection of Barium

Chemical Basis: Induced precipitation of lead sulfate from a masked solution. Lead sulfate dissolves easily in ammonium acetate solution containing acetic acid, forming a complex lead acetate. Consequently, no lead sulfate precipitates when sulfate ions are added to lead solutions containing considerable quantities of ammonium acetate and acetic acid. Accordingly, solutions which still contain free SO_4^{--} ions can be prepared by dissolving lead sulfate in ammonium acetate solution. If a solution of this kind is used to precipitate barium as $BaSO_4$, the precipitate contains not only barium sulfate, but also considerable quantities of lead sulfate. This is probably due to the formation of an adsorption compound of $BaSO_4$

plus $PbSO_4$, or possibly these two slightly soluble sulfates form mixed crystals. The precipitation of lead sulfate, which normally does not occur from solutions containing acetate, therefore is induced by the precipitation of barium sulfate. The formation of the extra quantity of precipitate raises the sensitivity of the barium sulfate reaction. Accordingly, barium solutions, which are so dilute that sulfuric acid produces no visible precipitate, or only after several hours standing, give a distinct turbidity immediately when an acetous solution of lead sulfate is added.

Procedure: The reagent is prepared by mixing 2 ml. of 10 per cent lead acetate solution with 2 ml. of 2 *N* sulfuric acid and then dissolving the precipitated lead sulfate by adding solid ammonium acetate to the suspension. A drop of the neutral or slightly acid test solution is treated with a drop of the lead sulfate reagent. The reaction can be carried out in a micro-test tube or on a watch glass (black glossy paper beneath). A more or less intense turbidity or white precipitate will form, depending on the quantity of barium present.

Application in the presence of other materials: The test for barium is not specific, since the coprecipitation of lead sulfate is also induced by the precipitation of strontium sulfate and calcium sulfate. Consequently, if an unknown mixture gives a positive reaction, the test gives no information beyond revealing the presence of alkaline earth metals.

2. Detection of Sulfate (Barium)

Chemical Basis: Irreversible tinting of barium sulfate precipitated in the presence of potassium permanganate. If barium sulfate is precipitated from a solution containing a considerable quantity of potassium permanganate, the precipitate will be deep red-violet. The color is due to an adsorption or inclusion of $KMnO_4$ in the crystal lattice of the nascent barium sulfate. Aged precipitated barium sulfate does not take up permanganate; thus substantiating the foregoing statement. The permanganate fixed on barium sulfate is quite resistant to reducing agents (H_2O_2, SO_2, $H_2C_2O_4$, etc.) which ordinarily react with it at once. Hence, the excess permanganate in the solution can be separately decolorized by reduction to manganous ion, and the violet tinted barium sulfate thus made visible. Since the barium sulfate precipitation is a test for either SO_4^{--} ions or Ba^{++} ions, this tinting reaction can be used for detecting either ionic species.

Procedure (Test for SO_4^{--}): Three drops of the solution to be tested are mixed with a drop of a saturated solution of potassium permanganate on a spot plate. A drop of this solution is placed on filter paper which has been soaked in 0.5 *N* barium chloride and then dried. The test paper is then placed for 7 to 10 minutes in an oven at 70°–80°C. The excess

barium chloride is removed by immersing the paper in water for one minute. It is then held briefly in a stream of tap water, and then placed in 1 *N* oxalic acid solution. A pink ring will remain if sulfate is present.

Identification Limit: 2.5 γ sulfuric acid.

Concentration Limit: 1:20,000.

Procedure (Test for Ba^{++}): Strips of filter paper are soaked in 0.5 *N* sodium sulfate and then dried. This sulfate paper can be kept on hand. Three drops of the solution to be tested are placed on a spot plate or in a microcrucible. A drop of saturated permanganate solution is added. An alternative method is to treat a drop of the test solution with a droplet of permanganate of approximately one third the volume. A drop of the violet solution is placed on dry sodium sulfate paper and then kept for 7 to 10 minutes in an oven at 70°–80°C. The original violet will disappear, and the paper will become brown because of the manganese dioxide resulting from the reaction of the cellulose and the permanganate. The sensitivity of the test reaction is somewhat decreased if the drying is prolonged. The dried spot paper is bathed in a solution of sulfurous acid until the last traces of manganese dioxide have faded. The complete reduction and removal of the dioxide and the excess permanganate require about 1 to 2 minutes. A violet fleck or ring, depending on the quantity of barium, will be seen on the perfectly white paper at the place where barium sulfate was formed.

Identification Limit: 5 γ barium.

Concentration Limit: 1:10,000.

The test can also be carried out in an Emich centrifuge tube. One drop of the test solution is mixed with 3 drops of a cold saturated solution of potassium permanganate. Several drops of dilute sulfuric acid are then added, and the color is discharged with a solution of sulfurous acid, added drop by drop. The violet barium sulfate precipitate is collected in the tip of the tube by centrifuging. It can be clearly seen there against a white background; if necessary, under a magnifying glass.

Identification Limit: 2.5 γ barium.

Concentration Limit: 1:20,000.

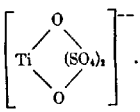
Application in the presence of other materials: The foregoing test is specific for the detection of sulfate ion. With reference to the test for barium, it should be noted that strontium sulfate becomes slightly tinted. Consequently, if strontium salts are to be tested for barium, a blank test should be made with samples of pure strontium salts. As little as 5 γ barium can be detected in the presence of 2500 γ strontium. Calcium sulfate does not take up permanganate. On the other hand, lead sulfate precipitated in the presence of permanganate behaves like barium precipitate.

3. Detection of Titanium

Chemical Basis: Induced precipitation of titanium by precipitation of zirconium arsenate. Zirconium salts react in acid solution with arsenic acid and immediately form slightly soluble zirconium arsenate. Under these conditions, titanium salts only react slowly, and the more acid and dilute the solution the less complete their reaction. If, however, zirconium and titanium are both present, almost all the titanium is coprecipitated with the zirconium arsenate. After the suspension is centrifuged and the precipitate dissolved in sulfuric acid, the titanium, which has thus been localized, can be identified by an appropriate reaction.

The test for titanium is a drop reaction only in so far as a drop of the test solution suffices. A dilute solution is prepared from this drop and an abundant zirconium precipitate can then be obtained from this dilute solution. If very dilute titanium solutions are involved from the start, the following procedure can be used. It still gives positive results in 10 ml. of a solution diluted 1:10,000,000. The test involves the pertitanic acid reaction. The very sensitive test with chromotropic acid described on p. 205 can also be used.

Procedure: Ten milliliters of the test solution are mixed with one drop of 1 per cent solution of zirconium salt, 20 drops of 20 per cent arsenic acid solution are added, and the mixture is warmed for a short time. After cooling, the precipitate is collected by centrifuging. The precipitate is dissolved in warm 6 *N* sulfuric acid and the solution is treated with 3 per cent hydrogen peroxide. If titanium is present, the solution turns yellow. The color is due to the formation of the complex yellow peroxodisulfato-titanium ion



This is formed by the reaction:

$$TiOSO_4 + H_2O_2 + H_2SO_4 = H_2O + H_2[TiO_2(SO_4)_2].$$

Application in the presence of other materials: The localizing procedure just described accomplished a selective separation of titanium from the accompanying materials which normally interfere with the identification of titanium by means of hydrogen peroxide. Small quantities of titanium can thus be detected in the presence of considerable iron, vanadium, and chromium, under conditions at which the normal hydrogen peroxide test fails.

Chapter V

QUALITATIVE ORGANIC ANALYSIS BY SPOT TESTS

Direct detection of certain organic compounds by spot reactions, utilizing methods that are as simple as the specific or selective detection of ions, has hitherto been possible only in isolated cases. In all likelihood this condition will continue. The possession of specific or selective tests would be no advantage in view of the many thousands of organic compounds, since, in practice, it would be impossible to deal in this fashion with unknown samples. Far more important than the detection of single compounds is the establishment of the presence or absence of certain characteristic constituents of organic compounds, either elements or definite groups. This information is entirely adequate for many purposes. For instance, if it has been proved that sulfur or nitrogen is not present, it is certain that the many organic groups containing these elements must also be absent. On the other hand, supplementary detection of an SO_3H or SH group in sulfur bearing compounds often gives adequate information as to the nature of the sample.

Characteristic groups of atoms can sometimes be detected directly by their behavior toward certain reagents. If the reaction products are colored or have other characteristic properties the procedure employed in these tests is quite analogous to that used for spot reactions of inorganic compounds. However, another line of attack must be used in the majority of cases. Certain groups of atoms are subjected to building up-, rearrangement-, or degradation reactions, quite like those employed in the preparation of organic compounds; they are thus transformed into compounds which either permit an analytical treatment *per se* because of their characteristic color or solubility, or react in a recognized manner with certain reagents. Tests requiring preliminary preparative or synthetic steps are, of course, more troublesome than the simple tests that can be applied to inorganic ions. They are also less reliable and definite because the formation, as well as the reactivity, of organic compounds is often extensively influenced by the presence of other groups in the molecule. The possibility of steric hindrance, as well as the presence or absence of acidic or basic groups, plays an important rôle. Therefore, the positive or negative response to a test for a certain group of atoms may not always be regarded as final and requiring no further consideration. The finding must be viewed as merely directive until the positive results of other tests corroborate the previous conclusion.

Organic syntheses and other procedures of preparative organic chemistry

for use in spot test analysis must be such as can be carried out with small quantities of the test material (1 or 2 drops or particles of solid). Consequently, only such procedures need be considered as are based on organic reactions taking place almost quantitatively. Fussy operations such as distillation, crystallization, etc., are not only time-consuming, but invariably involve considerable loss of material. Accordingly, qualitative analysis of organic materials with the aid of spot reactions always utilizes relatively simple procedures which can be carried out directly with the equipment used in inorganic spot testing.

A. Detection of Certain Elements in Organic Compounds

The presence of carbon and hydrogen can be taken for granted in all organic compounds and no special tests for them are necessary. The same is frequently true for oxygen, whose presence is simultaneously established by the recognition of groups containing this element. Sulfur and nitrogen are particularly important among the other non-metals that are usually encountered in organic compounds. It is often necessary to test for halogens, but not as often for phosphorus and arsenic. The light metals may occur as salts of the organic acids, the alkali metals being encountered more frequently than the alkaline earth metals. As a rule, it is not often necessary to test for the heavy metals excepting mercury. Mercury may be encountered both in the form of its salts and also directly linked to carbon.

The basic principle in testing for the non-metallic elements is the appropriate destruction of the organic skeleton and the formation of an inorganic compound which can then be recognized by the methods commonly employed in qualitative inorganic spot analysis.

1. Detection of Halogens, CN, CNS

Chemical Basis: Conversion into a volatile copper halide, etc. Organic compounds containing halogen (Cl, Br, I), CN, CNS when heated with cupric oxide produce volatile copper halides that confer a characteristic green or blue-green color on a non-luminous gas flame. This reaction succeeds with all classes of organic compounds but it must be remembered that certain nitrogenous substances (oxyquinolines, urea and thiourea, α-substituted pyridine derivatives, etc.) containing no halogen will color the flame if this procedure is used. These compounds, when decomposed by heat in the presence of copper oxide, form small quantities of volatile copper cyanide.

Fluorine, as is well known, often differs from the other halogens in its reactions. It does form CuF_2, but, in contrast to the other copper halides, this compound does not volatilize.

Procedure: A piece of copper wire, 1 mm. in diameter, is fastened into a glass rod; the end of the wire is then beaten out to form a spatula 2–3 mm. wide. The spatula is heated in the oxidizing flame and becomes coated with copper oxide. A little of the powdered sample or a drop of its solution (which should contain no free halogen acid) is gently evaporated to dryness on the spatula. The wire is then heated fairly strongly in the non-luminous flame of a burner, first in the inner zone and then in the lower part of the outer zone. The persistence of the blue or green color will vary with the halogen content. A platinum spatula is still better. A little copper oxide is mixed with the sample and the mixture heated on the tip of the spatula.

Sensitivity: The method gives positive results with 0.5 γ chloronitrobenzene, 0.25 γ iodoeosin.

2. Detection of Nitrogen

Chemical Basis: Conversion into ammonia and identification through formation of 2 $Ag \cdot MnO_2$. When organic compounds containing nitrogen and hydrogen are heated with lime, ammonia is formed. This can be detected easily. A very sensitive test for ammonia is based on its action with a neutral solution containing both manganous and silver nitrates. The OH^- ions react:

$$2\,Ag^+ + Mn^{++} + 4\,OH^- = 2\,Ag + MnO_2 + 2\,H_2O.$$

The insoluble reaction products are not formed as a mixture but as an adsorption system of free silver and manganese dioxide. This adsorption complex is intensely black and consequently serves to reveal traces of ammonia.

The test is conveniently made in a small glass tube. The gas evolved is allowed to react on paper soaked with the reagent solution. A grey or black fleck develops if ammonia is given off. It is well to add some manganese dioxide to the lime, as otherwise the combustion will be incomplete and tarry products will deposit on and darken the paper, leading to the false conclusion that ammonia has been evolved. Oxygen furnished by the manganese dioxide will insure complete combustion. A part of the ammonia will also be oxidized, but in view of the high sensitivity of this test, the loss of ammonia is not important.

Procedure: A little of the sample is mixed in a hard glass test tube with lime and manganese dioxide. Alternatively, a drop of the test solution is evaporated to dryness (preferably under reduced pressure) and the residue mixed with the lime and manganese dioxide. The open end of the tube is then covered with a piece of filter paper moistened with the silver-

manganese nitrate reagent solution (for preparation see p. 227). The tube is slowly heated to redness. A black or gray stain, depending on the amount of nitrogen present, appears on the paper. The stain turns blue when treated with benzidine acetate solution (preparation, see p. 142). The MnO_2-benzidene reaction is discussed on p. 141.

Sensitivity: This procedure gives positive results with: 2 γ sulfanilic acid; 1 γ *p*-nitrosophenol; 3 γ codein hydrochloride.

3. Detection of Sulfur

Chemical Basis: Conversion into alkali sulfide and identification through the iodine-azide reaction. Alkali sulfides are formed when organic sulfur compounds are heated with metallic sodium or potassium. Since very small quantities of sulfides can be detected by the liberation of nitrogen in the iodine-azide reaction described on p. 163, this affords a sensitive and specific means of testing for organically combined sulfur. The iodine-azide reaction is carried out in a solution acidified with acetic acid; addition of cadmium acetate prevents any escape of hydrogen sulfide.

Procedure: A little of the powdered sample is placed in a small hard glass tube whose end is blown out to a bulb. If the sample is liquid a micro-drop is evaporated to dryness, preferably under slightly reduced pressure by connecting the tube to a suction pump. A piece of potassium the size of a pin head is introduced on a small glass rod and any sample on the walls of the tube can be stroked down with the metal. The tube is then heated carefully, starting at the open end, until the potassium has melted and mixed with the sample. Finally, the tube is heated to redness for a short time and then placed at once in a micro-test tube containing 5 drops of water. The particles of glass and carbon need not be removed. One drop of cadmium acetate solution (20 per cent) and then 1 or 2 drops of 20 per cent acetic acid are added and, when cold, 1 or 2 drops of iodine-azide solution (preparation see p. 165). If a sulfur compound was present in the sample, bubbles of nitrogen will be seen.

Sensitivity: The procedure gives positive results with: 0.3 γ thiourea; 1.2 γ sulfanilic acid.

4. Detection of Phosphorus and Arsenic

Chemical Basis: Conversion into phosphate (arsenate) by heating with lime. Organic compounds or materials containing phosphorus or arsenic form heat-resistant tertiary calcium phosphate or arsenate when heated with lime. The phosphate will react with a nitric acid solution of ammonium molybdate to produce ammonium phosphomolybdate, which can be identified by the very sensitive benzidine reaction (see p. 146). The

arsenate can be reduced to the element by a solution of stannous chloride containing concentrated hydrochloric acid:

$$2\ As^{+++++} + 5\ Sn^{++} \rightarrow 2\ As + 5\ Sn^{++++}$$

The arsenic can be recognized as a black precipitate or coloration.

Procedure (phosphorus): A few grains of the powdered sample are placed in a hard glass micro-test tube or, alternatively, a drop of the test solution is evaporated to dryness in the tube. The evaporation is best accomplished under slightly reduced pressure by gently warming the tube while it is attached to a pump. A few milligrams of lime are added and mixed with the sample. The tube is then heated, gently at first, and finally at red heat for a few moments. While still hot, it is placed in 2 drops of distilled water in a depression of a spot plate. The tube breaks on touching the water. The mixture on the spot plate is treated with 2 drops of concentrated nitric acid, which dissolves the calcium salts. A drop of ammonium molybdate solution (preparation see p. 147) is then added. The spot plate is heated gently on an asbestos mat and, after cooling, a drop of benzidine solution (preparation see p. 142) is added, followed by 1 or 2 drops of saturated sodium acetate solution. If the mixture turns blue, the presence of phosphorus is indicated, provided arsenic is absent.

Procedure (arsenic): A few grains of the powdered sample are placed in a hard glass micro-test tube, or a drop of the test solution is evaporated to dryness in the tube, preferably by warming and using the suction pump. A few milligrams of lime are mixed with the sample and the tube is heated gently at first, and finally to redness for a few minutes. The contents of the tube are allowed to cool completely and are then dissolved in a few drops of concentrated hydrochloric acid. (No particles of carbon will remain if the ignition has been complete.) Two drops of a freshly prepared solution of stannous chloride in concentrated hydrochloric acid (35 per cent) are added and the mixture warmed. A black precipitate or brown turbidity will appear if arsenic is present. The turbidity can be made more visible by shaking out the mixture with ether. The dark particles will then be seen at the ether-water interface.

Sensitivity: These procedures will give positive results with: 1 γ 1-phenyl-1-chloroethylene-2-phosphinic acid; 12 γ chloroarsanilic acid; 12 γ nitroarsanilic acid; 10 γ sodium *N*-acetoarsanilate.

B. Detection of Characteristic Groups of Atoms

The immense number of organic compounds would present a vast chaos of materials if it were not possible to arrange them in classes (alcohols, carboxyl compounds, amines, etc.) whose members are characterized by the presence of certain groups of atoms. The most important function

of qualitative organic analysis is, therefore, to provide reliable means of identifying such typical groups. These groups are not only essential in the classification of organic compounds but, in many cases, they also determine their chemical and physical properties. Only a few of the characteristic groups of atoms can be detected directly, that is, by direct reaction with suitable reagents capable of forming products which are colored or otherwise so characteristic that they can be recognized easily by spot reactions. It is usually necessary to use preliminary preparative measures (condensation, oxidation, and so forth) to transform the sample into materials which, in turn, are susceptible to the action of appropriate reagents.

It is not advisable to test unknown materials for the presence of typical groups with undue haste. As in qualitative inorganic analysis, an orientation should be obtained by a series of preliminary tests which often give valuable indications as to which groups should be tested for. The following tests will be found useful for directing the operator to a judicious choice:

Behavior on heating: Concentration of solutions to discover whether solid or liquid materials predominate; testing to determine whether the sample melts, decomposes, burns completely, or leaves a residue or ash.

Solubility in water: Strong organic acids and bases, as well as the alkali salts (in many cases, the salts of light metals also) of organic acids, and salts of mineral acids with organic bases, are soluble in water. The free acids or bases are precipitated on adding mineral acid or alkali to the water solutions of these salts (precipitate or separation of oil).

Solubility in organic solvents: Organic compounds containing hydroxyl groups and likewise the salts of organic acids or bases are, as a rule, not soluble in organic liquids immiscible with water. Free organic acids or bases, as well as organic compounds having no acid or base character, are often soluble in ether.

Testing materials not soluble in water: Solubility in fixed alkalies indicates weakly acidic compounds (—COOH, —SH, —OH groups, etc.). Solubility in dilute hydrochloric acid points to the presence of nitrogen bases. Neutral materials remain unchanged when tested with acids and bases. Consequently, if the ether solution of an organic compound is shaken out with dilute alkali or dilute acid, the resulting salts of organic acids or bases pass into the water layer. It is possible to segregate acid, basic, and neutral compounds from mixtures by this procedure.

Recrystallization and determination of melting point: Recrystallization from suitable solvents, followed by determinations of the melting point, often furnish valuable indications as to whether pure materials or mixtures are at hand. Sometimes separations can be obtained by recrystallizing.

The foregoing tests are not absolutely trustworthy; they are merely

directives which often facilitate further investigation of unknown organic materials. They can be made with very small quantities of the sample. Frequently the question to be answered by qualitative organic tests is the mere detection of certain types of compounds. In such cases preliminary tests are not necessary and the operator can immediately proceed to determine whether the group tests give positive or negative results.

1. Detection of Nitroso Compounds (—NO Group)

Chemical Basis: Reaction with sodium pentacyano-ammine-ferroate. Nitroso compounds form highly colored complex compounds with the water-soluble complex iron cyanides: sodium pentacyano-aqua-ferroate $Na_3[Fe(CN)_5H_2O]$ and sodium pentacyano-ammine-ferroate $Na_3[Fe(CN)_5NH_3]$.

The color reaction depends on the exchange of the water or ammonia molecule for a molecule of the nitroso compounds:

$$Na_3[Fe(CN)_5NH_3] + RNO \rightarrow NH_3 + Na_3[Fe(CN)_5RNO].$$

A few thioaldehydes and thioketones also react with these cyano salts to give a blue color. Hydrazine and some of its derivatives give a red or violet color. This interference may be prevented by adding a few drops of formaldehyde; formhydrazones are produced, which do not react with these reagents.

Procedure: A drop of the test solution is mixed on a spot plate with a drop of a freshly prepared (1 per cent) solution of sodium pentacyano-ammine-ferroate. After some time an intense green or, more rarely, a violet color develops.

Sensitivity: This method gives positive results with: 0.15 γ *p*-nitroso-dimethylaniline (emerald green); 1.0 γ α-nitroso-β-naphthol (dark green); 2.5 γ isonitroso-acetylacetone (brown-lilac); 3.0 γ isonitroso-acetophenone (green).

2. Detection of Nitro Compounds (—NO_2 Group)

Chemical Basis: Electrolytic reduction to nitroso compounds and identification with sodium pentacyano-ammine-ferroate. The color reaction of nitroso compounds with sodium pentacyano-ammine-ferroate described in the preceding paragraphs can be applied to the detection of nitro compounds if the nitro group is reduced to the nitroso group. The reduction can be carried out electrolytically in a drop of the test solution.

Procedure: A drop of the alcoholic or aqueous test solution is placed in a micro-crucible and mixed with a drop of freshly prepared (1 per cent) solution of sodium pentacyano-ammine-ferroate and a drop of 4 *N* sodium

hydroxide. If the addition of alkali produces a coloration, a drop of sodium sulfate solution (5 per cent) should be used instead as the electrolyte. The current is passed through the mixture, using a nickel wire as cathode and a lead wire as anode. The current is furnished either by a flash light battery or a 4 volt storage battery. The electrolysis should be continued for at least ten minutes; for small quantities of the sample, half an hour. During the electrolysis, the liquid becomes colored. The color, which deepens on standing, is usually green, more seldom, violet. No alteration in the pale yellow color will be observed in a blank test with sodium sulfate, but with alkali there is a slight deepening in the shade.

Sensitivity: This procedure gives positive results with: 1.5 γ nitrobenzene (dark violet); 2 γ *o*-chloronitrobenzene (green); 15 γ *p*-chloronitrobenzene (green); 0.4 γ *o*- and *p*-nitrophenol (dark green); 3 γ *o*-nitrocinnamic acid (green).

3. Detection of Primary and Secondary Alcohols

Chemical Basis: Conversion into alkali xanthate and identification by molybdate. Primary and secondary alcohols in the presence of alkali hydroxides react with carbon disulfide to produce alkali xanthates:

$$ROH + CS_2 + NaOH \rightarrow CS(OR)(SNa) + H_2O.$$

All alkali xanthates react with molybdate, in the presence of mineral acids, to form violet compounds soluble in chloroform. These colored materials are addition compounds of molybdic anhydride and xanthic acid of the general formula $MoO_3[SC(SH)OR]_2$.

The molybdate-xanthate reaction is very sensitive; even small quantities of primary and secondary alcohols, after conversion into the corresponding xanthates, can be detected with the aid of acidified molybdate solutions.

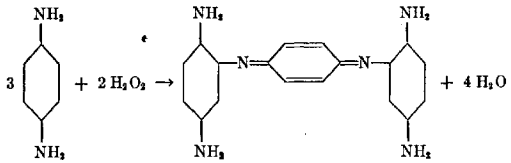
Since esters are partially saponified to alcohols under the conditions of the test, they react similarly. Compounds containing the $—CH_2 \cdot CO \cdot CH_2—$ group also react with carbon disulfide and alkali. They form orange-red compounds which, on treatment with molybdate, produce chocolate-brown precipitates, insoluble in chloroform, and hence interfere with the detection of small quantities of alcohols.

Procedure: A drop of the test solution, in ether if possible, is shaken for about 5 minutes in a test tube with a drop of carbon disulfide and several centigrams of solid sodium hydroxide. One or two drops of 1 per cent ammonium molybdate solution are then added. As soon as the alkali has dissolved, the solution is carefully acidified with 2 *N* sulfuric acid and shaken with 2 drops of chloroform. If alcohols were present, the chloroform solution becomes violet.

Sensitivity: Positive results are given by: 1 mg. of ethyl, methyl, or isobutyl alcohol; 0.5 mg. of cyclohexanol; 0.1 mg. of phenyl ethyl alcohol.

4. Detection of Aldehydes (—CHO Group)

Chemical Basis: Catalytic acceleration of the oxidation of p-phenylenediamine by hydrogen peroxide. In acid or neutral solution, *p*-phenylenediamine (I) is oxidized by hydrogen peroxide to a black compound (II), known as Bandrowski's base.



(I) (II)

The velocity of the oxidation is appreciably increased by aldehydes. This peroxydase reaction may thus be applied to the detection of aldehydes if the test is carried out in certain definite concentrations of reagent and acids, in which the uncatalyzed reaction is retarded whereas the catalytic action of the aldehydes is not appreciably affected.

In neutral solution, all aldehydes produce a black color or precipitate and preceded by other transitory colorations [persisting a little longer in the case of aromatic aldehydes]. Aliphatic aldehydes react the same in both acid and neutral solution, but most aromatic aldehydes form a yellow precipitate or color which lasts for some time in acid solution. This difference is useful in distinguishing between aliphatic and aromatic aldehydes. The nitriles, aldehyde-ammonia and bisulfite compounds behave like their parent aldehydes. The oximes of aldehydes are somewhat less reactive. Ketones do not react.

Procedure: A drop of the reagent solution (2 per cent *p*-phenylenediamine), 2 drops of 2 *N* acetic acid, and 2 drops of 3 per cent hydrogen peroxide are mixed with a drop of the test solution on a spot plate. If aldehydes are present, a color develops. With large amounts, the color appears at once, but only after a short time when small quantities of aldehyde are present. It is always advisable to carry out a blank test on a drop of water and also a parallel test omitting the acetic acid, as some aldehydes react more rapidly in acid and others in neutral solution. A yellow color in acid solution indicates the presence of an aromatic aldehyde.

Sensitivity: Positive results are given by:

	Acid	*Neutral*
0.02 γ Formaldehyde	green	black
	black	black
0.25 γ Acrolein	green	red
	black	black
3.5 γ Benzaldehyde	yellow	brown
		black
0.8 γ Salicylaldehyde-bisulfite	orange	yellow
		black
50 γ Hexamethylenetetramine	violet	violet
	black	black

5. Detection of Methyl Ketones (—CO_2CH_3 Group)

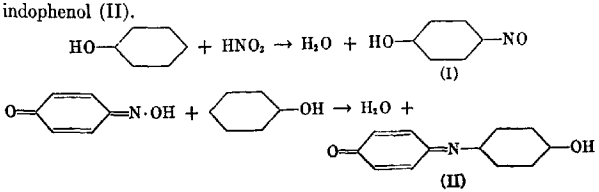
Chemical Basis: Reaction with sodium nitroprusside. Acetone reacts with sodium nitroprusside and alkali to produce an intense red-yellow color; this changes to pink-violet when acidified with acetic acid. Under these conditions an alkaline solution of nitroprusside is decolorized, but the mechanism of this color reaction is not clear. Other methyl ketones react similarly to acetone, whereas ordinary ketones give no reaction.

Procedure: A drop of the aqueous or alcoholic test solution is mixed in a microcrucible with a drop of 5 per cent sodium nitroprusside solution and a drop of 30 per cent sodium hydroxide. A slight color usually develops after a short time. One or two drops of glacial acetic acid are then added. A red or blue color indicates the presence of a methyl ketone.

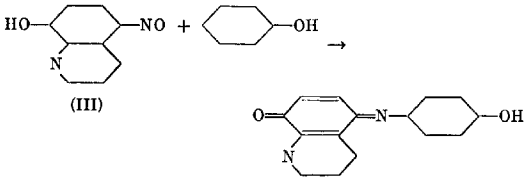
Sensitivity: Positive results are given by: 2 γ acetylacetone (purple); 4 γ acetoacetic ester (orange); 10 γ acetone (pink); 15 γ acetone dicarboxylic acid (violet).

6. Detection of Phenols (—$\overset{\diagdown}{\underset{\diagup}{C}}$—OH Group)

Chemical Basis: Conversion into colored indophenols. If a phenol, whose *para* position is unoccupied, is treated with a solution of nitrite in concentrated sulfuric acid, i.e., with nitrous acid, a characteristic color is obtained. This is due to the formation of an indophenol, which is a quinoid compound. The production of the dye results from the fact that the phenol is nitrosated in the *para* position (I) and the resulting nitroso compound, in its tautomeric quinone form, condenses with unchanged phenol, and forms the indophenol (II).



The first step required for the formation of indophenol, namely, the nitrosation, can be obviated if a solution of a stable nitroso compound in concentrated sulfuric acid is used as the reagent. 5-Nitroso-8-hydroxyquinoline (III) is suitable for this purpose. It reacts with phenol, for instance as follows:



(III)

Sensitivity: The following give positive results: 1 γ phenol (dark brown); 2 γ resorcinol (red-violet); 5 γ *o*-nitrophenol (green-yellow).

7. Detection of Thioketones and Mercaptans (=CS and $-\overset{\diagdown}{\underset{\diagup}{C}}-SH$ Groups)

Chemical Basis: Catalysis of the sodium azide-iodine reaction. The reaction

$$2\,NaN_3 + I_2 = 2\,NaI + 3\,N_2$$

normally proceeds very slowly. It is catalyzed not only by inorganic sulfides, thiosulfates, and thiocyanates (see p. 164), but also by organic compounds that contain the groups =C=S or $-\overset{\diagdown}{\underset{\diagup}{C}}-SH$. Other organic sulfur compounds such as thioethers (R—S—R), disulfides (R—S—S—R) [with the exception of diacyl disulfides (R—CO—S—S—CO—R)], sulfones, ($R—SO_2—R$), sulfinic acids ($R—SO_2H$), and sulfonic acids ($R—SO_3H$), or salts of the two last named acids, have only a very slight effect, if any, on the reaction.

Initiation of the iodine-azide reaction can be ascertained either by the formation of bubbles of nitrogen or by the disappearance of the iodine. Both effects can be used for detecting the presence of the active =CS and $-\overset{\diagdown}{\underset{\diagup}{C}}-SH$ groups (Procedures I and II). Procedure II is the more sensitive, but care must be exercised when interpreting its results because other compounds, under these conditions, may oxidize iodine and thus consume it.

Compounds containing the =C=S and $-\overset{\diagdown}{\underset{\diagup}{C}}-SH$ groups may be differentiated by the fact that the latter are converted to disulfide

$$2\,R-SH + I_2 \rightarrow R-S-S-R + 2\,HI$$

which does not react with iodine-azide solution. Consequently, if a sample gives a positive result with the iodine-azide solution, another sample should be treated with iodine and heated for a short time with sodium acetate and then tested again with the iodine-azide solution. If a positive result is obtained again, the =C=S group is present.

Procedure I: A drop of a solution of the test substance in water or an organic solvent is mixed on a watch glass with a drop of the iodine-azide solution and observed for any evolution of nitrogen. The reagent is a solution of 3 g. of sodium azide in 100 ml. of 0.1 *N* iodine solution. Carbon disulfide should not be used to dissolve the sample as it reacts with the iodine-azide solution (see p. 163). Either a solid or liquid compound (after removal of the solvent) may be tested directly with the reagent. In this case it is particularly easy to note a positive reaction, even when very small amounts of the material are used.

Procedure II: A drop of the test solution is placed on filter paper and the organic solvent is removed by a current of warm air. The fleck is outlined with pencil, and a drop of the sodium azide solution containing only a little iodine is added. The starch in the paper combines with the iodine. Consequently, in the absence of compounds containing active sulfur groups, a round blue fleck appears. On the other hand, if the fleck contains a material which initiates the iodine-azide reaction, the iodine in this area will be consumed and the formation of the blue iodine-starch will be prevented in this area. Therefore, a blue circular zone surrounding a white inner circle appears. If the sample is a solid it is not necessary to put it into solution; several particles can be placed on filter paper and spotted with the iodine-azide solution. The reagent used in this procedure is a 2 per cent solution of sodium azide in 0.01 *N* iodine.

Sensitivity: Positive results are obtained with 0.005 γ thiourea; 0.04 γ potassium xanthate; 0.03 γ rubeanic acid.

8. Detection of Sulfonic Acids (—SO_3H Group)

Chemical Basis: Formation of sulfite by alkaline fusion and identification of sulfurous acid. If aromatic and aliphatic sulfonic acids are fused with caustic alkalies, the sulfonic acid group is replaced by hydroxyl, and alkali sulfite is formed. For instance, the reaction in the case of benzene sulfonic acid is:

$$C_6H_5SO_3H + 3\ NaOH = C_6H_5ONa + Na_2SO_3 + 2\ H_2O.$$

As even minute quantities of sulfite, or sulfur dioxide liberated on acidification, can be detected by the induced oxidation of green nickelous to black nickelic hydroxide (see p. 162), a test for sulfonic acid groups can be based on the alkaline fusion coupled with the ensuing sulfite reaction.

Sulfinic acids as well as sulfones also produce sulfite when fused with alkalies. Consequently, the test will reveal the presence of $-SO_2H$, $-SO_2H$, and $-SO_2$ groups, provided divalent sulfur compounds are not also present. The latter easily produce alkali sulfide in this fusion and there is considerable oxidation of sulfide to sulfite. Furthermore when the melt is acidified, hydrogen sulfide is liberated; this acts on the nickel hydroxide and forms black nickel sulfide. A preliminary test will quickly reveal any formation of alkali sulfide in the fusion. The melt need merely be taken up in water and tested with sodium nitroprusside solution. A violet color indicates the presence of sulfide and therefore of bivalent sulfur in the sample. Under such circumstances, the procedure given here cannot be used directly, but should be modified as follows: A water suspension of the sample is warmed with solid mercuric oxide and then filtered or centrifuged. Mercury mercaptides and thioketones remain undissolved. The filtrate or centrifugate contains mercury sulfonate which can be subjected, after evaporation, to the alkaline fusion.

Procedure: An ignition tube with a 2 to 3 ml. bulb is used. A small quantity of the solid material, or of the residue left after evaporating a drop of solution in the ignition tube, is heated with a granule of sodium hydroxide over a small flame until the contents of the tube just melt. After cooling, the melt is dissolved in 2 drops of water and treated with 1 or 2 drops of concentrated hydrochloric acid (test with litmus paper) and the walls of the tube are rinsed with water. After the edge of the tube is wiped carefully, a piece of filter paper smeared with nickel hydroxide paste is placed across the tube. The lower end of the tube is held for several minutes in hot water to hasten the evolution of the sulfur dioxide. If the green nickel hydroxide turns black or gray, sulfite is present. When very small quantities of sulfite are involved, it is better to spot the nickel hydroxide, after it has reacted with the sulfur dioxide, with an acetic acid solution of benzidine. A blue will appear if traces of nickelic hydroxide are present. The nickel hydroxide paste is prepared by treating nickel chloride with sodium hydroxide and then washing the $Ni(OH)_2$ free from alkali.

Sensitivity: The following give positive results: 3 γ α-sodium naphthalene disulfonate; 6 γ potassium methyl mercaptide; 6 γ benzenesulfinic acid; 6 γ sulfonal.

9. Detection of Amines ($-NH_2$ and $=NH$ Groups)

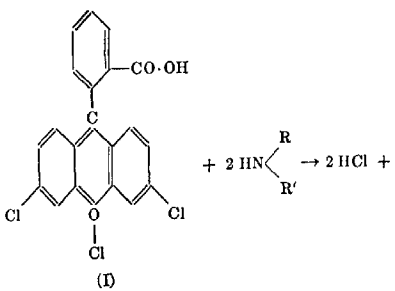
Chemical Basis: The following tests for primary and secondary aliphatic and aromatic amines are based on the ability of such materials to condense with fluorescein chloride to form dyes of the rhodamine series (II). The condensation is easily accomplished by melting the hydrochlorides of the

base with fluorescein chloride (or its oxonium chloride (I)) and anhydrous zinc chloride. The colors, in natural and ultraviolet light, thus obtained differ characteristically according to the type of the amine involved. Consequently, not only $-NH_2$ and $=NH$ groups can be detected and differentiated, but it can also be determined whether these groups were linked to aliphatic or aromatic radicals.

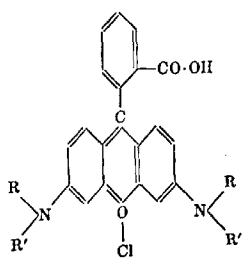
Pyrrole derivatives condense through the cyclic $=NH$ group of the pyrrole ring

to form rhodamine dyes. These can be characteristically distinguished from other rhodamines by their special fluorescence.

The formation of rhodamine dyes can be represented by the general scheme:



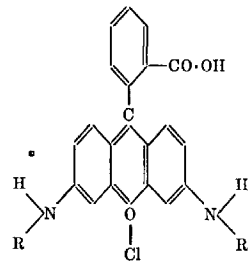
(I)



(II)

(a) Detection of Primary Aliphatic Amines

If primary aliphatic amines or their salts are fused with fluorescein chloride, pink dyes with yellow-green fluroescence are formed. They have the general structure:



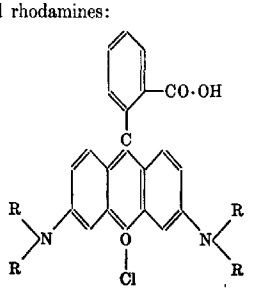
These dialkylated rhodamines exhibit an intense yellow-green fluorescence both in daylight and in ultraviolet light. The rhodamines produced from acid amides (see p. 186) and from ammonium salts fluoresce similarly.

Procedure: A drop of the test solution acidified with hydrochloric acid is evaporated to dryness in a test tube. The residue is mixed with a little fluorescein chloride (tip of spatula) and twice this quantity of zinc chloride. The test tube is heated in an air bath (large iron crucible or aluminum block) at 250° to 260°C. until the zinc chloride is completely fused. After cooling, the melt is dissolved in 10 per cent alcoholic hydrochloric acid. The solution shows a green-yellow fluorescence if primary aliphatic amines were present. If only small quantities of amine are involved, it is necessary to view the fluorescence under a quartz lamp.

Sensitivity: The procedure gives positive results with: 10 γ methylamine; 10 γ benzylamine; 20 γ glycocoll ester; 20 γ hydrazine sulfate.

(b) Detection of Secondary Aliphatic Amines

The fusion of secondary aliphatic amines with fluorescein chloride produces tetra-alkylated rhodamines:



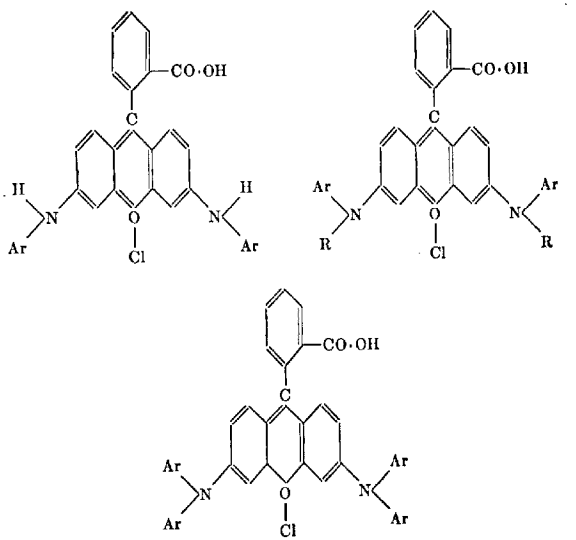
which are red in acid solution. The solutions have an orange-red fluorescence. The fluorescence colors therefore make it possible to distinguish between primary and secondary amines, since only the former give reaction products fluorescing green-yellow.

Procedure: The method is that employed for primary aliphatic amines.

Sensitivity: The following give positive results: 4 γ diethylamine; 4 γ piperidine; 20 γ aceturic (acetyl glycine) ester.

(c) Detection of Aromatic Amines

Primary, secondary, and tertiary aromatic amines, containing a methyl group, produce the following types of dyes when fused with fluorescein chloride:



The alcohol solutions of these dyes, acidified with hydrochloric acid, are intensely red-violet but, in contrast to the solutions of the rhodamines formed from aliphatic amines, they exhibit no fluorescence. Consequently, the presence or absence of a fluorescence makes it possible to distinguish between aliphatic and aromatic amines. The benzal derivatives react like their parent amines.

Procedure: The method is that employed for primary aliphatic amines.

Sensitivity: The following give positive results: 5 γ aniline; 2 γ naphthylamine; 4 γ benzidine; 8 γ diphenylamine.

10. Detection of Acid Amides and Nitriles

Acid amides when fused with fluorescein chloride behave like the primary aliphatic amines. The same is true of nitriles, since they are saponified to acid amides. The resulting yellow fluorescing rhodamine dyes have not been isolated as yet. In all likelihood compounds analogous to those formed from primary amines are produced.

Procedure: The test is carried out by the procedure given for the primary aliphatic amines but care must be taken that the test solution is neutral when it is evaporated, as the amides may be saponified in acid solution. Dyes with a yellow fluorescence are formed.

Sensitivity: The method gives positive results with: 20 γ acetamide; 20 γ benzamide; 40 γ phthalimide.

11. Detection of Pyrrole Derivatives

Pyrrole derivatives, when fused with fluroescein chloride, form yellow-brown dyes which fluoresce blue in ultraviolet light. They can thus be distinguished from all other amines.

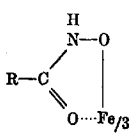
Procedure: The method is the same as that used for other amines. The test solution may be neutral or alkaline during the evaporation.

Sensitivity: The following give positive results: 40 γ pyrrole; 12 γ indole; 30 γ carbazole.

12. Detection of Carboxylic Acids and Their Derivatives

Carboxylic acids, esters, and anhydrides can be converted readily into hydroxamic acids of the general formula R·CO·NHOH. The procedure is different and characteristic for each class of these compounds.

All hydroxamic acids, in acid solutions, produce a red or violet color with ferric chloride. This color reaction is due to the acid —CO·NHOH group, which is present in all hydroxamic acids. With ferric ion, inner complex salts are formed, having the structural formula:



This formation of ferric hydroxamate is used as a test for carboxylic acids and their derivatives.

(a) Detection of Carboxylic Acids (—COOH Group)

Chemical Basis: Conversion into ferric hydroxamate. Carboxylic acids cannot be converted directly into hydroxamic acids. It is necessary to produce the acid chloride which gives the hydroxamic acid when tested with hydroxylamine and alkali. The underlying reactions are:

$$R\cdot CO\cdot OH + SOCl_2 \rightarrow R\cdot COCl + SO_2 + HCl.$$
$$R\cdot COCl + NH_2OH + NaOH \rightarrow R\cdot CO(NHOH) + NaCl + H_2O.$$
$$3\ R\cdot CO(NHOH) + FeCl_3 \rightarrow 3\ HCl + Fe[R\cdot CO(NHO)]_3.$$

Procedure: A drop of the acid test solution, evaporated to dryness in a micro-crucible, or a minute portion of the solid is used. Two drops of thionyl chloride are added and the mixture evaporated almost to dryness to convert the carboxylic acid into its chloride. Two drops of a saturated alcoholic solution of hydroxylamine hydrochloride are added and then a sufficient number of drops of strong alcoholic caustic alkali to make the liquid alkaline to litmus paper. The reaction takes place on warming. The mixture is then acidified with a few drops of 10 per cent alcoholic hydrochloric acid and treated with 0.5 per cent ferric chloride solution. The acidity of the solution should be confirmed with litmus. The reaction mixture becomes brown-red or dark violet if a carboxylic acid was present in the sample.

Sensitivity: Positive results are obtained with: 12 γ monochloroacetic acid; 15 γ aminoacetic acid; 11 γ oleic acid; 12 γ anthranilic acid.

(b) Detection of the Esters of Carboxylic Acids (—COOR Group)

Chemical Basis: Conversion into ferric hydroxamate. Esters of carboxylic acids react with hydroxylamine in the presence of alkali, and form hydroxamic acids:

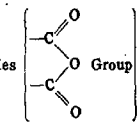
$$R\cdot CO\cdot OR + NH_2OH = R\cdot CO(NHOH) + ROH.$$

Lactones, which can in fact be viewed as inner esters, behave like esters. The hydroxamic acids produce colored inner complex ferric salts (see p. 186).

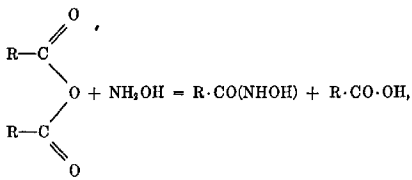
Procedure: A drop of the ether solution of the ester is treated in a porcelain micro-crucible with one drop each of a saturated alcohol solution of hydroxylamine hydrochloride and of saturated alcoholic caustic potash. The mixture is heated over a micro-burner until a slight bubbling indicates that the reaction has started. The contents of the crucible are then acidified with 0.5 *N* hydrochloric acid and a drop of 1 per cent ferric chloride solution is added. A violet color appears, its intensity depending on the quantity of ester present.

Sensitivity: The following give positive results: 11 γ ethyl formate;

10 γ ethyl urethane; 2.5 γ phenyl acetate; 2.5 γ methyl salicylate; 6 γ coumarine.

(c) **Detection of Carboxylic Acid Anhydrides**  **Group**

Chemical Basis: Conversion into ferric hydroxamate. Carboxylic acid anhydrides react with hydroxylamine to form hydroxamic acids:

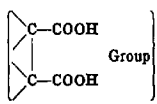


$$+ NH_2OH = R\cdot CO(NHOH) + R\cdot CO\cdot OH,$$

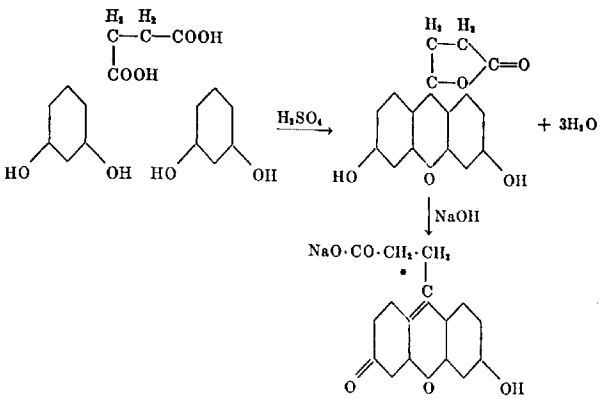
which give soluble colored inner complex ferric salts (see p. 186).

Procedure: A drop of the ether solution of the sample is placed in a porcelain micro-crucible. One or two drops of the reagent solution are added and the mixture evaporated to dryness over a micro-burner. A few drops of water are added and a violet or pink develops, depending on the amount of anhydride that was present. The reagent consists of 0.5 per cent alcoholic solution of ferric chloride acidified with a few drops of concentrated hydrochloric acid and then saturated by heating with hydroxylamine hydrochloride.

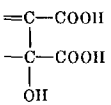
Sensitivity: The method gives positive results with: 5 γ acetic anhydride; 5 γ succinic anhydride; 6 γ benzoic anhydride; 10 γ camphoric anhydride.

(d) **Detection of 1,2-Dicarboxylic Acids**  **Group**

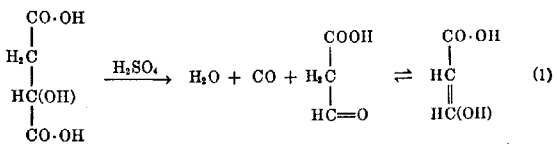
Chemical Basis: Conversion into dyes of the fluorescein series by fusion with resorcinol. Dicarboxylic acids with the carboxyl groups in the 1,2 or 1,4 positions (one —COOH group may be substituted by a $—SO_3H$ group) or their derivatives, such as esters, anhydrides or imides, form dyes of the fluorescein type on melting with resorcinol. These give a vivid greenish yellow fluorescence in alkaline solution. The reaction proceeds:

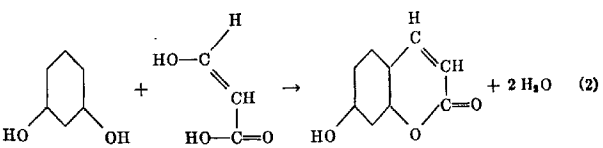


The 1,2-dicarboxylic acids with a free —OH group adjacent to the carboxyl group:



react in a different manner and give off CO by the action of concentrated sulfuric acid on the resorcinol melt, to form semialdehydes of malonic acid or its homologues (Equation 1). These condense with resorcinol to umbelliferone or its homologues (Equation 2):





The umbelliferones formed are almost colorless, but give an appreciable fluorescence in daylight. Under the mercury vapor lamp the fluorescence is a brilliant blue.

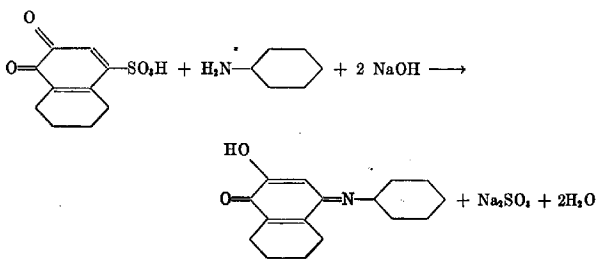
Procedure: A few milligrams of the test substance are placed in a microcrucible, or a drop of test solution is evaporated to dryness, mixed with a little freshly sublimed resorcinol and a few drops of pure concentrated sulfuric acid and heated to 130°C. for 5 minutes, either on an asbestos plate, or still better, on an aluminum block containing depressions to hold 2 crucibles and a thermometer. When the reaction is complete the crucible and contents is dropped into water in a 50 cc. beaker to dissolve out the products. The solution is made alkaline with sodium hydroxide. In the presence of substances containing the groups just described, a fluorescence occurs, which is especially bright in ultraviolet light.

A blank test should always be carried out, for, when the temperature has exceeded 130°C., a blank can show a fluorescence which is green by daylight and greenish blue in ultraviolet light. Apparently, at the higher temperature the resorcinol decomposes partially and gives rise to dicarboxylic acids.

Sensitivity: The method gives positive results with: 5 γ tricarballylic acid; 5 γ succinic acid or anhydride; 5 γ phthalic acid; 5 γ saccharin.

13. Detection of Reactive $-CH_2$ and $-NH_2$ Groups

Chemical Basis: Condensation with 1,2-naphthoquinone-4-sulfonate. Deeply colored quinoid condensation products are formed when 1,2-naphthoquinone-4-sulfonic acid reacts in alkaline solution with compounds containing two removable hydrogen atoms attached to a carbon or a nitrogen atom. The orthoquinoidal reagent is transformed by these reactions into a paraquinoidal condensation product. The reaction occurs as shown in the following equation in which aniline is taken as an example.



The test may only be applied to confirm orientation as the reactivity of the $—CH_2$ and $—NH_2$ groups is influenced by the other atomic groups in the molecule of the compound in question. For example, aniline derivatives with strongly negative groups, such as trinitraniline and tribromaniline, will no longer condense in this way. Negative groups in the *ortho-* and *para-* positions cause much more interference with the reaction than negative groups in the *meta-* position.

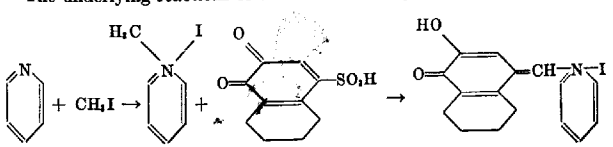
Procedure: A small quantity of the solid or a drop of its solution is treated in a microcrucible with 2 drops of sodium naphthoquinone sulfonate solution and then made alkaline with 2 drops of 0.5 *N* sodium hydroxide. When the mixture is acidified with 2 *N* acetic acid the color changes; sometimes a precipitate forms. It is best to use freshly prepared reagent. It consists of a saturated solution of sodium 1,2-naphthoquinone-4-sulfonate in 50 per cent alcohol.

Sensitivity: Positive results are given by 1.2 γ malonic ester; 6 γ nitraniline (meta); 0.7 γ methylamine; 0.6 γ piperidine; 0.6 γ semicarbazide.

14. Detection of Tertiary Ring Bases

Chemical Basis: Quaternisation of the nitrogen and condensation with sodium 1,2-naphthoquinone-4-sulfonate. As stated on p. 190 organic compounds containing two replaceable hydrogen atoms react with naphthoquinone sulfonic acid in alkaline solution to form colored quinoid compounds. The $—CH_3$ or $—C_2H_5$ groups, which do not react with naphthoquinone sulfonic acid in the compounds CH_3I or C_2H_5I, may be activated by attaching the alkyl iodide to a tertiary nitrogen base or oxonium compound. The quaternary compound that results, reacts at once with naphthoquinone sulfonic acid to give colored (red, red-violet, or green) compounds. As the color reaction is very definite, the formation of the quaternary compound during the reaction with napthoquinone sulfonic acid can be applied as a test for cyclic tertiary bases and for oxonium compounds. It is of course essential that $—CH_2$ and $—NH_2$ groups should not be present, as they react with the naphthoquinone sulfonic acid (see p. 190).

The underlying reactions of the test can be expressed:



Similar equations can be written for other alkylation agents such as dimethyl sulfate, ethyl bromide, ethyl iodide and *p*-bromo-toluene sulfonic acid methyl ester. The formation of the quaternary compound proceeds best with methyl iodide and dimethyl sulfate.

Procedure: A small amount of the test substance (either the solid or a drop of its solution) is mixed in a tall micro-crucible with 5 or 6 drops of methyl iodide or dimethyl sulfate and heated to gentle boiling on an asbestos plate. Substances hard to convert to the quaternary form should be heated for some hours with methyl iodide in a closed capillary placed in a water bath at 100°C. Two or three drops of a saturated solution of sodium naphthoquinone sulfonate are added to the quaternary compound and the mixture made alkaline with 0.5 *N* sodium hydroxide. The appearance of color or change of color indicates that the test is positive. On acidifying with 1 *N* acetic acid there is a change of color.

Sensitivity: Positive results are given by: 12 γ pyridine; 12 γ α-picoline; 25 γ quinoline; 25 γ dimethylpyrone.

C. Detection of Specific Organic Compounds

A definite identification of certain organic compounds by characteristic reactions has only been possible in isolated cases up to now. The principal reason is that the majority of organic compounds with typical groups have homologs. These are derived from a parent form of a simple structure by more less extensive substitution by groups which are inactive from the analytical standpoint. For instance, in the aldehyde series, R—CHO, the typical group is —CHO and R— may be an aliphatic or an aromatic radical. Because of the aldehyde group, all these compounds are more or less active reducing agents. They are also characterized by the activity of the O= atom. These characteristics are exhibited most sharply by the parent compound formaldehyde, $\begin{matrix} H \\ H \end{matrix}\!\!>\!C{=}O$. This aldehyde occupies a special position and can be identified by specific tests. Aliphatic and aromatic aldehydes exhibit differences which can be revealed by analytical methods, for the aliphatic or aromatic radical affects the chemical behavior of the aldehyde group. However, the individual members of the extensive classes of aliphatic or aromatic aldehydes no longer exhibit differences great enough to be distinguished chemically. In such instances a search for specific reactions is useless. The same is true for many other organic compounds with typical groups. As a rule, special reactions are only available for the parent forms of the simplest possible structure, but not for the large number of homologous compounds. Consequently, specific

tests are possible only for compounds which have no homologs or whose homologs are very sluggish in reaction. If complicated organic compounds are susceptible to a rearrangement or a degradation to the most simply constructed organic parent compound, a special reaction for the latter may then be used as a test for the original compound. In view of these facts, the establishment by chemical methods of the presence of typical groups is often all that can be accomplished. Any further identification of chemical compounds can be undertaken only after they have been isolated. Then melting points or other physical constants can be determined, or a quantitative analysis can be made.

The following sections describe several tests which can be regarded as characteristic for single compounds.

1. Detection of Formaldehyde

Chemical Basis: Reaction with chromotropic acid. When formaldehyde (or compounds producing formaldehyde) are heated with chromotropic acid in the presence of concentrated sulfuric acid a violet-red color appears. The chemistry of this color reaction is not known.

The following give no reaction: acetaldehyde, propionaldehyde, butyraldehyde, isobutyraldehyde, isovaleraldehyde, crotonaldehyde; oenanthol, chloralhydrate, glyoxal, and aromatic aldehydes. Glyceraldehyde, furfural, arabinose, fructose, and sucrose give yellow colors. Other sugars, acetone, and carboxylic acids do not react. High concentrations of furfural give a red color.

Procedure: A drop of the test solution is mixed in a test tube with 2 ml. of 72 per cent sulfuric acid (100 ml. water and 150 ml. concentrated sulfuric acid). A little solid chromotropic acid is added and the tube is heated in a water bath at 60°C. for 10 minutes. If formaldehyde is present a bright violet develops. A blank is advisable for comparison if small amounts are suspected.

Identification Limit: 0.14 γ formaldehyde.

Concentration Limit: 1:360,000.

2. Detection of Methyl Alcohol

Chemical Basis: Oxidation to formaldehyde and identification with chromotropic acid. Methyl alcohol may be oxidized to formaldehyde in dilute phosphoric acid solutions by treatment with permanganate. The formaldehyde can then be detected by the selective chromotropic acid reaction just described. Under the conditions prescribed here, ethyl alcohol is oxidized only to acetaldehyde so that the test may be used for the

detection of traces of methyl alcohol in the presence of ethyl alcohol. For example, 5.3 γ methyl alcohol may be detected in a drop of 40 per cent ethyl alcohol, which implies a proportion limit of 1:1150. Glycerol, arabinose, fructose, lactose, and sucrose give yellow colors, and furfural a brown.

Procedure: A drop of the test solution is mixed in a test tube with a drop of dilute phosphoric acid solution (10 ml. of 50 per cent acid diluted to 100 ml.) and a drop of potassium permanganate solution (5 per cent) and left for one minute. A little solid sodium bisulfite is added, with shaking, until the mixture is decolorized. If any brown precipitate of the higher oxides of manganese remains undissolved, a further drop of phosphoric acid should be added and a little more sodium bisulfite. When the solution is quite colorless 4 ml. of 72 per cent sulfuric acid and a little finely powdered chromotropic acid are added. The mixture is well shaken and then heated to 60° C. for 10 minutes. A violet color which deepens on cooling indicates the presence of methyl alcohol.

Identification Limit: 3.5 γ methyl alcohol.

Concentration Limit: 1:13,600.

3. Detection of Formic Acid

Chemical Basis: Reduction to formaldehyde and identification with chromotropic acid. Formic acid is readily reduced to formaldehyde by magnesium and hydrochloric acid. The formaldehyde may then be identified by the chromotropic acid test (p. 193). Glucose interferes owing to a partial breakdown to formic acid; no other sugars or acids interfere.

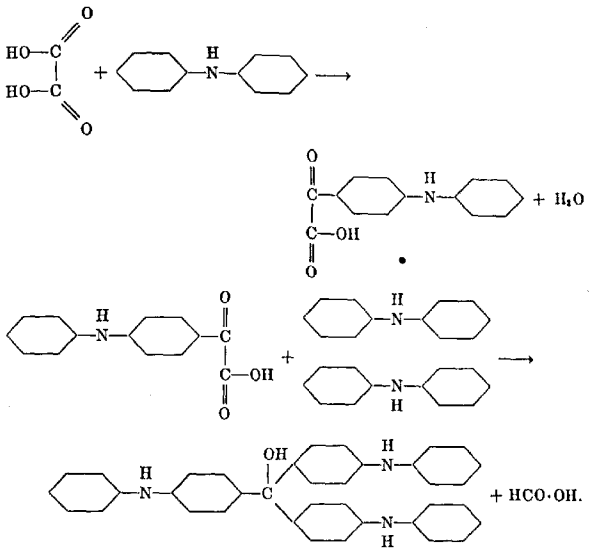
Procedure: A drop of the test solution is mixed in a test tube with a drop of 2 *N* hydrochloric acid. Magnesium powder is added until no further gas is liberated. Three milliliters of 72 per cent sulfuric acid and a little chromotropic acid are introduced and the tube is heated in a water bath at 60° C. for ten minutes. If formic acid is present, a violet color develops.

Identification Limit: 1.4 γ formic acid.

Concentration Limit: 1:20,000.

4. Detection of Oxalic Acid

Chemical Basis: Formation of aniline blue by fusion with diphenylamine. A triphenylmethane dyestuff, diphenylamine blue or aniline blue, is formed when oxalic acid and diphenylamine are melted together. This dye is also formed when insoluble oxalates are warmed with diphenylamine and syrupy phosphoric acid. The following equations represent the synthesis of aniline blue from oxalic acid and diphenylamine:



Formic, acetic, propionic, tartaric, citric, succinic, dihydroxy-maleic, benzoic, phthalic, tricarballylic, glycolic, or glyoxylic acid do not react under the conditions of the experiment. The formation of aniline blue in the reaction with diphenylamine is very selective for oxalic acid.

Procedure: A crystal of the sample substance (a solution must be evaporated to dryness) is melted with a little diphenylamine in a micro-test tube over a free flame. On cooling, the melt is taken up in a drop of alcohol, when a blue color indicates the presence of oxalic acid. Under the same conditions a blank test remains colorless.

When oxalic acid is to be detected in a mixture containing other anions precipitated by $CaCl_2$ (sulfate, sulfite, fluoride, tartrate, or pyruvate ions) it is advisable to proceed as follows. The acetic acid solution is treated with $CaCl_2$, the precipitate collected on a filter or in a centrifuge tube and freed from water either by drying or by washing with alcohol and ether. A small amount of the precipitate is mixed with diphenylamine in a dry test tube, syrupy phosphoric acid is then added and the tube is heated over the free flame. This causes formation of calcium phosphate and liberation of oxalic acid, which can condense to aniline blue with the diphenylamine, as indicated by the coloration of the phosphoric acid. This

is decolorized on cooling, but on taking up the melt in alcohol a brilliant blue appears. The excess diphenylamine is precipitated by adding water and colored light blue by absorption of the dye. A blank test treated in the same way gives a pure white precipitate of diphenylamine. The dye can be extracted from the aqueous solution with ether, which increases the sensitivity of the reaction. On long standing the ethereal extract again separates into two layers. The blue-violet product is formed most densely on the ether-water interface.

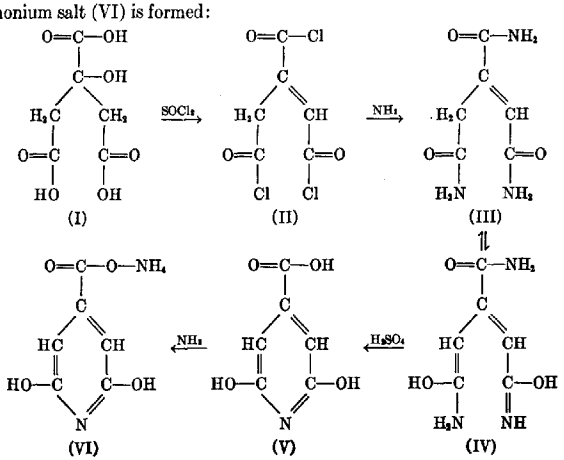
Identification Limit: 5 γ oxalic acid.

Concentration Limit: 1:10,000.

5. Detection of Citric Acid

Chemical Basis: Conversion into citrazinic acid. The ammonium salt of 2,6-dihydroxypyridine-4-carboxylic acid (V) (citrazinic acid) has a deep blue fluorescence in aqueous solution. Citric acid can easily be converted to citrazinic acid by a new method, starting with a drop of the test solution. The presence of citric acid can be detected by the blue fluorescence of the ammonium salt of the citrazinic acid.

The stages in the conversion to citrazinic acid are the following: The treatment of citric acid (I) with thionyl chloride forms aconitic acid chloride (II); this is converted into the triamide (III) on boiling with ammonia; this compound has a tautomeric form (IV) and in this form gives off ammonia on heating with 80 per cent sulfuric acid; the ring closes, and the acid amide group in the middle is saponified. The compound thus formed is citrazinic acid (V). On the addition of ammonia the fluorescent ammonium salt (VI) is formed:



The test is specific for citric and aconitic acid; malic acid and tartaric acid do not react.

Procedure: A drop of the test solution is evaporated to dryness in a microcrucible and the residue treated with 4 drops of thionyl chloride and fumed. About 8 drops of concentrated aqueous ammonia are then added and the mixture boiled over a microburner until about 2 drops of liquid remain in the crucible. On cooling, 6 drops of concentrated sulfuric acid are added and heating is continued to dense fumes. The contents of the crucible are poured into a test tube and rendered ammoniacal. An intense blue fluorescence appears in ultraviolet light if the sample contained citric acid.

Identification Limit: 1 γ citric acid.

Concentration Limit: 1:50,000.

6. Detection of Glycerol

Chemical Basis: Conversion into acrolein. Glycerol when heated with a dehydrating agent, such as potassium bisulfate, is converted into the unsaturated aldehyde acrolein

$$CH_2(OH)\cdot CH(OH)\cdot CH_2(OH) \rightarrow CH_2{:}CH\cdot CHO + 2\ H_2O.$$

On the addition of an aqueous solution of sodium nitroprusside containing a little piperidine, acrolein gives a blue color that turns violet-red with alkalies. The chemistry is not known; up to the present it has not been possible to isolate a compound of definite formula from the components necessary for the color reaction, although the acrolein may be substituted by other aldehydes, and the piperidine by secondary aliphatic amines.

The acrolein formed by removal of water from glycerol may alternatively be detected by the reaction with dianisidine, in which Schiff's base is formed with loss of water.

Glycerides (animal and plant fats) also form acrolein on heating with potassium bisulfate. The test may therefore be applied to the examination of technical or natural products for traces of fat or glycerol where the presence of these is undesirable.

Procedure: A small amount of the test substance or a drop of the test solution is placed in the hard glass tube described on p. 52 (Fig. 30) and mixed with finely powdered potassium bisulfate. A piece of filter paper moistened with the reagent is placed over the open end of the tube and covered with the glass cap. The acrolein formed after long heating colors the test paper a deep gentian blue. On treating the paper with 2 *N* sodium hydroxide the blue portion changes to the color of peach blossom. The reagent is a freshly prepared 1 per cent solution of sodium nitropruss-

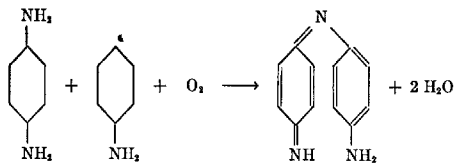
ide containing a drop of piperidine, or a saturated solution of *o*-dianisidine in glacial acetic acid.

Identification Limit: 5 γ glycerol.

Concentration Limit: 1:10,000.

7. Detection of *p*-Phenylenediamine

Chemical Basis: Oxidation to an indamine dye. When *p*-phenylenediamine is mixed with an oxidizing agent in the presence of aniline, in slightly acid solution, a blue-green indamine dye is formed immediately:



Derivatives of *p*-phenylenediamine behave similarly. The reaction may be applied to detect the presence of *p*-phenylenediamine (poison!) in furs, hair dyes, etc.

Procedure: A drop of the acetic acid test solution is mixed with a drop of aniline water (1 drop of aniline in 50 ml. water). A few crystals of potassium persulfate are added. A blue-green appears at once; it is dark or pale, depending on the quantity of phenylenediamine present.

Identification Limit: 0.5 γ *p*-phenylenediamine.

Concentration Limit: 1:100,000.

CHAPTER VI

APPLICATION OF SPOT REACTIONS TO STUDIES OF ROCKS AND MINERALS

The identification of minerals and rocks by the classical methods of mineralogy and petrography always requires the determination of the chemical and physical properties of the specimen. The data thus obtained constitute the bases of the identification procedures. Chemical analysis often plays a dominant rôle in this type of investigation since measurements of physical properties alone frequently do not suffice for satisfactory mineral and rock determinations. Spot analysis sometimes presents the possibility of considerably simplifying such determinations, especially by shortening the chemical tests. Sensitive spot reactions are available which can be used to establish the presence or absence of characteristic metallic or non-metallic constituents quickly and definitely. Furthermore, reactions which can be carried out as spot reactions sometimes clear up questions as to how characteristic components are combined. Such knowledge likewise facilitates the identification and classification of minerals and rocks. The application of spot analysis to this special field is recent. The results obtained thus far have shown that it is possible to identify and determine minerals and rocks quickly, simply, and also definitely with quite small quantities of the specimen. The tedious operations, even including quantitative analyses, otherwise necessary can sometimes be obviated. Occasionally, mineralogical and petrographic chemical tests by spot reactions can be made directly on the specimen without removing a test portion. The reaction can be carried out, for example, on a chosen part of the surface, on a crystal face, or on a section. As a rule, tests are made with small quantities of pulverized samples which can be scratched off with a needle without perceptibly harming the sample. If colored and stable reaction products are obtained from the tests, permanent mounts can be made. They may give information as to the distribution of certain materials, or they may be used for comparison purposes. It is often possible to apply spot reactions successfully in the field, that is, masses of rock, ore, etc., may be tested *in situ*.

1. Differentiation between Magnesite and Dolomite (Breunnerite)

Determination of the Presence of Dolomite in Limestone

Magnesite is pure magnesium carbonate, while dolomite is a double carbonate of magnesium and calcium of the formula $MgCO_3 \cdot CaCO_3$. Both are members of the calcite group of rhombohedral carbonates; the other physical properties of these two materials are also very much alike.

Consequently, a clear differentiation between them was formerly possible only by establishing the content of calcium, preferably by a quantitative analysis. Breunnerite, a mineral quite similar to dolomite, is also a double carbonate of magnesium; it has the formula $MgCO_3 \cdot FeCO_3$. In this case also, a differentiation from magnesite was only possible by establishing the presence of considerable quantities of iron.

A rapid method of distinguishing between magnesite and dolomite is not only an asset to the mineralogist, but it also has technical value as magnesite is the most important raw material for the preparation of metallic magnesium. Consequently, when magnesite deposits are worked, it is often important to differentiate between magnesite and the less valuable dolomite deposits. It is frequently useful to determine whether a limestone is composed mainly of calcite or of dolomite, and it is possible to do this in the field. The procedure given here can also be used to determine the presence of magnesium carbonate or dolomite in limestone; it requires very small quantities of the sample.

Chemical Basis: The different behavior of magnesite and dolomite toward diphenylcarbazide. Magnesium hydroxide, oxide, and carbonate form violet compounds of unknown composition with diphenylcarbazide. Probably these are adsorption compounds similar to those described on page 104 in the discussion of the test for magnesium. Magnesite and ignited magnesite (magnesium oxide) therefore react immediately when tested with a warm solution of diphenylcarbazide. A pulverized specimen or even larger particles of the solid turn red-violet at once and the color is retained even after thorough washing. In contrast, double carbonates such as dolomite and breunnerite, remain unaltered, so that it is possible to distinguish quickly between dolomite (also breunnerite) and magnesite. The double carbonates of magnesium, namely dolomite and breunnerite, do not react, probably because they are to be regarded as salts of a hypothetical magnesium-carbonato acid; their formulas are $Ca[Mg(CO_3)_2]$ and $Fe[Mg(CO_3)_2]$ respectively. The magnesium is masked in these double carbonates, that is, it is no longer present as magnesium carbonate, which can react with diphenylcarbazide, but is a constituent of an unreactive complex anionic group $Mg(CO_3)_2^{--}$.

Procedure: The reagent is a freshly prepared solution of diphenylcarbazide in alcohol. Several drops of dilute alkali should be added to it just before making the test. One or two drops of the hot reagent are placed in a depression of a spot plate and a particle, the size of a pin head, of the rock is added. After 5 minutes the colored solution is drawn off with a pipette, replaced by hot water, and this type of washing is continued until the water is no longer tinted. If the specimen was magnesite, or if it contained magnesite inclusions, a red-violet product remains. If the

sample was dolomite, or if the magnesium was in dolomitic combination (breunnerite) no tinting occurs. The identification of the magnesium can be made as follows: A grain (pin-head size) of the sample is heated strongly on a platinum crucible lid. The characteristic dolomitic linkage is thus destroyed and reactive magnesium oxide, together with calcium oxide, is formed. The magnesium oxide can then be identified by the method described previously.

The procedure can also be used for the direct detection of slight quantities of magnesium (down to 0.01 per cent) in limestones, provided the magnesium is not combined dolomitically. In dolomitic limestones, or those containing dolomite, the magnesium can be detected by the diphenylcarbazide reaction after the specimen has been ignited.

The *identification limit* of the magnesium-diphenylcarbazide reaction is 0.5 γ magnesium in drops.

Suggested test materials: Magnesite, dolomite, breunnerite, dolomitic limestone, tooth powder.

2. Differentiation of Calcite and Aragonite

Calcite and aragonite are two modifications of calcium carbonate. Chemically they are completely identical and can be distinguished directly only through their crystalline forms (hexagonal and rhombic, respectively). Calcite is the stable modification at all temperatures, aragonite the unstable form. This difference is evidenced in the solubilities of the two varieties. The determination of the nature of a given sample of calcium carbonate is often important. For instance, the deposits (tufa) laid down by hot springs are usually aragonite, and the products, such as mussel shells, secreted by organisms have likewise been found to be aragonite.

Chemical Basis: Differentiation through differences in basicity. The two modifications of calcium carbonate differ slightly with respect to solubility in water and rate of solution. Aragonite is more soluble than calcite. A suspension of calcium carbonate presents the following series of equilibria:

$$CaCO_3 \text{ (solid)} \rightleftharpoons CaCO_3 \text{ (dissolved)} \rightleftharpoons Ca^{++} + CO_3^{--}.$$

The carbonate ion hydrolyzes:

$$CO^{--} + 2\,H_2O \rightleftharpoons H_2CO_3 + 2\,OH^-$$
$$\text{or}$$
$$CO_3^{--} + H_2O \rightleftharpoons HCO_3^- + OH^-.$$

Since aragonite is more soluble than calcite, solid aragonite, when suspended in or placed in contact with water, delivers OH^- ions quicker and in greater numbers than calcite. A neutral solution of Mn^{++} plus Ag^+ salts is an excellent reagent for OH^- ions since a black precipi-

tate is formed immediately, even at low concentrations of hydroxyl ions, because of the reaction:

$$2\,Ag^{+} + Mn^{++} + 4\,OH^{-} = 2\,Ag + MnO_2 + 2\,H_2O.$$

Accordingly, if a solid or pulverized specimen of aragonite or calcite is brought into contact with the reagent solution, a gray to black deposit forms on the surface of aragonite almost immediately or, in any case, within about 2 minutes. Calcite, in contrast, remains unchanged at first and becomes colored only very slowly, in accordance with its lower solubility and lesser production of OH^- ions.

Procedure: The reagent is prepared by adding 1 g. of solid silver sulfate to a solution of 11.8 g. $MnSO_4 \cdot 7H_2O$ in 100 ml. of water. The solution is heated to boiling, allowed to cool, and the undissolved material filtered off. One or two drops of dilute sodium hydroxide are added, and after 1 to 2 hours the precipitate is filtered off. The solution is stable if it is stored in brown bottles. A tiny grain of the powdered mineral is spotted with a drop of the reagent solution on a spot plate or on a watch glass placed on white paper. If the specimen turns dark within 2 minutes, aragonite is present.

This differentiating reaction can also be carried out on thin sections. For instance, even the thinnest stratifications of aragonite and calcite are revealed in sections of stalactites by the blackening of the aragonite aggregates. A microscopic picture of this kind is just as sharp as those obtained by dyeing threads in textiles.

Suggested test materials: Precipitated chalk, aragonite, calcite, calcareous sandstone, mussel shells, tooth powder.

3. Differentiation of Gypsum and Anhydrite

Calcium sulfate occurs naturally as gypsum, $CaSO_4 \cdot 2H_2O$ (monoclinic) and as anhydrite, $CaSO_4$ (orthorhombic). Both serve as raw materials for the production of calcium compounds. Gypsum, in addition, is used in the manufacture of plaster casts, statuary, etc.

It is possible to distinguish these two forms of calcium sulfate through the fact that gypsum loses water when heated. They also differ in density and hardness. The differentiation by dehydration is definite only if the specimens are pure and contain no extraneous moisture. The test fails of course with moist products, or else a preliminary drying is necessary.

Chemical Basis: Difference in reaction rate on treatment with a solution of sodium carbonate. All compounds occurring with and without water of crystallization follow the rule that the hydrated form is more soluble and dissolves more quickly than the anhydrous form. Gypsum, the hydrated form, accordingly is more soluble than anhydrite; at 100° C.,

0.167 per cent gypsum dissolves, in contrast to 0.067 per cent anhydrite. There are marked differences in the solubilities at room temperature also, and consequently gypsum and anhydrite react at different rates with various water solutions of reagents, the reaction with the hydrated form always being the faster. An excellent reagent that reveals the differences in the reaction rates and therefore distinguishes gypsum from anhydrite is a solution of sodium carbonate, colored red with phenolphthalein. The solution reacts:

$$CaSO_4 + Na_2CO_3 = CaCO_3 + Na_2SO_4,$$

with both gypsum and anhydrite. The consumption of the alkaline sodium carbonate leads to the discharge of the red indicator color. Since gypsum is the more soluble form of calcium sulfate and reacts faster, under like conditions it discharges the color of the red sodium carbonate solution more rapidly than anhydrite. This fact may be used to distinguish the two forms of calcium sulfate.

Procedure: The reagent is a 4 per cent solution of sodium carbonate. Three drops of 2 per cent solution of phenolphthalein in alcohol are added. Very fine particles of the finely pulverized specimens are placed in two adjacent depressions of a spot plate and moistened with 2 or 3 drops of the red soda solution. On stirring with a platinum wire, the color lightens distinctly after 1 to 2 minutes with gypsum and is completely discharged after 4 to 5 minutes. The corresponding times with pulverized anhydrite are 15 minutes and 40 minutes.

The difference in the times required for discharge of the color can also be seen if one or two drops of the soda solution are placed on larger fragments. However, this method of distinguishing the two varieties is much less distinct than the test made with the pulverized specimens.

Suggested test materials: Gypsum, fibrous gypsum, anhydrite.

4. Detection of Chromium in Rocks, Steels, and Other Technical Materials

Most of the chromium in the earth's crust was segregated during the primary crystallization of the magma as chrome iron ore, $FeO \cdot Cr_2O_3$. Small quantities also occur in highly basic rocks, particularly in olivines, magnesium silicates, such as serpentine, etc. A slight chromium content is characteristic of many rocks. The detection of chromium is important when testing materials such as alloys, chrome steels, chrome platings, refractories, pigments; also in the detection of chrome tanned leather, the identification of chromium mordants on textiles, and of gelatins, glues, etc., hardened with bichromate.

Chemical Basis: Conversion into chromate and identification with diphenyl-

carbazide. No reaction of trivalent chromium is nearly so sensitive and selective as that of sexavalent chromium (chromate) into which metallic chromium, as well as all trivalent chromium compounds, can be easily converted. The chromate can be formed by either wet or dry methods. Ordinarily the latter are used. The solid specimen (or evaporation residue) is fused with an alkaline oxidizing agent:

$$Cr_2O_3 + 3\ Na_2O_2 = 2\ Na_2CrO_4 + Na_2O.$$

The chromium is detected by adding diphenylcarbazide in the presence of sulfuric acid. Even traces of chromate give a violet-red. The composition of this colored compound is not known.

The *identification limit* of the chromate-diphenylcarbazide reaction is 0.02 γ chromium in drops.

Examination of Rocks

A particle, the size of a pin head, of the specimen is ground to a fine powder in a small agate mortar. It is then mixed with 4 times its bulk of a mixture of equal parts of sodium-potassium carbonate and sodium peroxide (or sodium-potassium carbonate and potassium chlorate). If a glowing platinum wire is plunged once or twice into this oxidizing mixture, enough will be taken up to form a clear bead when melted. Any chromium will be converted to chromate. The cooled melt is placed in a depression of a spot plate, dissolved in 1 or 2 drops of sulfuric acid (1:1) and a drop of freshly prepared 1 per cent alcoholic solution of diphenylcarbazide introduced. If the specimen contained chromium, a pink to violet color appears, depending on the quantity of chromium. A blank test is necessary only if very small quantities of chromium are involved, or if an old reagent solution is used, since this often is yellow to pink.

Examination of Steel

The surface of the specimen is cleaned with emery paper. Two or three drops of a mixture of equal parts of nitric acid, sulfuric acid (1:1), and water are placed on this prepared surface. After about three minutes, a drop is taken off by means of a fine pipette, placed in a porcelain crucible, evaporated, and the residue gently ignited. A little sodium peroxide (tip of spatula) is introduced and the mixture fused until no more bubbles form. The solidified cooled melt is acidified with sulfuric acid (1:1) and tested for chromate with diphenylcarbazide solution.

Examination of Leather

A small quantity (less than 1 mg.) of the leather to be examined for chrome tanning is removed with a razor blade. In the case of shoes and

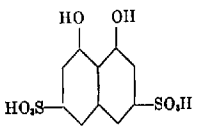
other finished articles, the sample should be taken from the inner side because leather dyes and dressings sometimes contain chromium, which obviously would lead to false conclusions. The particles of leather are ashed in a porcelain microcrucible. A little sodium peroxide is added to the cold residue and the mixture is heated to quiet fusion. The further treatment is as in steel.

Suggested test materials: Chromite, chrome mica (about 15 per cent Cr_2O_3), chrome steel, chromium plated iron, chrome tanned leather.

5. Detection of Titanium in Minerals, Technical Products, etc.

Titanium, as accompanying element, is practically always present in many siliceous rocks such as clay, kaolin, bauxite, etc. It is the essential constituent of ilmenite, $FeO \cdot TiO_2$, the most important raw material (in addition to rutile, TiO_2, and arizonite, $Fe_2O_3 \cdot 3TiO_2$) for the production of ferro-titanium and the pigment, titanium white, TiO_2. Glass frequently contains small amounts of titanium, and the identification of certain kinds of glass is made easier by testing for titanium. The pigments are used in face powders and creams, paper, soap, linoleum, artificial silk (to remove luster), rubber, and in drawing and printers' inks. Consequently, the detection of titanium is an important part of the technical examination of many materials.

Chemical Basis: Color reaction with chromotropic acid. The sodium salt of chromotropic acid, 1,8-dihydroxy-naphthalene-3,6-disulfonic acid,



in strong sulfuric acid solution, gives a violet color with titanium salts, titanium hydroxide, and titanic acid. The composition of the reaction product is not known; it is probably an inner complex salt, whose formation involves the —OH groups of the reagent.

The *identification limit* of the titanium-chromotropic acid reaction is 0.7 γ Ti in drops.

Procedure: A little (tip of knife blade) of the finely pulverized specimen is placed in a microcrucible and heated with several drops of concentrated sulfuric acid until dense fumes are evolved. After cooling, a granule of solid chromotropic acid is added and the mixture warmed briefly. A violet color appears if titanium is present; the intensity depends on the quantity.

If small amounts of titanium are to be detected in silicates, such as

glass, it is better to fume several milligrams of the finely powdered specimen in a platinum spoon with 2 or 3 drops of hydrofluoric acid. The greater portion of the silica is thus removed as SiF_4; the metals originally present as silicates are converted to fluorides. The latter are now converted to sulfates by warming with 2 or 3 drops of concentrated sulfuric acid. The contents of the spoon are then washed into a test tube containing a little concentrated sulfuric acid. Several grains of chromotropic acid are added and the mixture warmed again.

Suggested test materials: Titaniferous minerals (ilmenite, rutile); silicates with very slight titanium content (clay). Pigments: titanium white and, for comparison, lithopone (titanium-free).

6. Detection of Zinc in Minerals

Zinc occurs in minerals as oxide, silicate, or sulfide. Oxide minerals include zincite (ZnO); franklinite (Zn, Mn, Fe)O·$(FeMn)_2O_3$; smithsonite ($ZnCO_3$); hydrozincite ($Zn[OH]_2 \cdot ZnCO_3$). The most common zinc silicate is calamine ($2\ ZnO \cdot SiO_2 \cdot H_2O$); willemite ($2\ ZnO \cdot SiO_2$) is also fairly common. The most important zinc ore is the sulfide (zinc blende, wurtzite). Zinc sulfide is often associated in considerable quantities with galena (PbS).

Chemical Basis: Formation of zinc ferrocyanide from molybdenum ferrocyanide. If an alkali molybdate and an alkali ferrocyanide are brought together in acid solution, an amorphous red-brown precipitate is formed. This can be regarded as an addition compound of MoO_3 and hydroferrocyanic acid. Zinc salts react with this compound and produce an insoluble acid-resistant white zinc ferrocyanide:

$$H_4[MoO_3 \cdot Fe(CN)_6] + 2\ Zn^{++} = Zn_2Fe(CN)_6 + MoO_3 + 4\ H^+.$$

Consequently, if a drop of an acidified solution of a zinc salt is placed on filter paper impregnated with brown molybdenum ferrocyanide, a white fleck is formed.

Silver, lead, and cadmium salts react like zinc. However, the zinc test is not impaired by these ions if a solution of zinc sulfate is used (inactive $PbSO_4$ is formed), or if considerable quantities of sodium chloride were added beforehand to the test solution. Under these conditions, inactive silver chloride is formed and the Cd^{++} ions are converted into the inactive complex $CdCl_4^{--}$ ions (masking). The test for zinc in minerals must be made in various ways according to whether the zinc is present as oxide, silicate, or sulfide. Accordingly, the findings by the various procedures will reveal the manner in which the zinc is combined.

The *identification limit* of the zinc test on molybdenumferrocyanide paper is 0.5 γ zinc.

Procedure: The mineral to be tested is finely pulverized. Small quanti-

ties of the powder are taken for the following tests. The molybdenum ferrocyanide paper is prepared: Filter paper is bathed for several minutes in a solution containing 0.3 g. $(NH_4)_2MoO_4$ and 0.2 g. $K_4Fe(CN)_6$ in 100 ml. of water. After draining, the paper is dipped in 3 *N* acetic acid; brown molybdenum ferrocyanide precipitates in the paper. After thorough washing with water, the paper is dried in a current of warm air. The paper is stable.

Oxide. The powder is stirred with several drops of dilute sulfuric acid in a porcelain crucible and then warmed until dense fumes of sulfur trioxide are evolved. After cooling, several drops of water are added. Drops of the suspension are removed with a glass capillary and placed on the reagent paper. If zinc is present, a white fleck is formed on the brown paper.

Sulfide. The powder is ignited continuously in a porcelain crucible until the odor of sulfur dioxide has disappeared. The residue is cooled and treated as in the preceding paragraph.

Silicate. The powder is placed in a platinum crucible and fumed off twice with several drops of sulfuric acid plus hydrofluoric acid. The silicates are thus converted to sulfates. The mass is cooled and treated as in the oxide.

Suggested test materials: Zincite, franklinite, zinc blende, galena, calamine.

7. Differentiation of Siliceous Rocks and Minerals

I. Detection of the Presence or Absence of Potassium

Next to oxygen, silicon is the most widely distributed element in the earth's crust. The great family of silicate rocks may be divided into two main classes: (a) anhydrous and (b) hydrous silicates. The relation of silica to the basic oxide or oxides along with the nature of the basic oxide(s), the crystal form, density, optical properties, etc. determines the further division within these classes. In many cases a definite distinction is only possible on the basis of chemical analyses combined with due consideration of the physical data. Consequently, the identification of silicates by the classical methods is one of the most difficult problems of mineralogy and petrography.

The determination of the presence or absence of potassium can give valuable clues in the identification of silicates. Many groups and subgroups of the anhydrous and hydrous silicates contain materials that are otherwise quite similar to the related silicates, but they differ from these by containing considerable quantities of potassium. Examples are:

(a) *Anhydrous silicates:*

1. Felspars (di- and polysilicates): Potassium and sodium felspars are differentiated.

2. Meta silicates: Only the leucites contain potassium (or cesium).
3. Ortho silicates: Only certain members of the nepheline group contain potassium.

(b) *Hydrous silicates:*

1. Zeolites: Only members of certain groups contain potassium.
2. Micas: Potassium and sodium mica are differentiated.
3. Serpentines and Talcs: Only certain species contain potassium.

The differentiation of silicates is important not only in the solution of mineralogical and petrographic problems, but may be of direct technical importance, as certain silicates are the raw materials for the production of metals, metal salts, and other commercial products. For instance, hexagonal beryl ($3\ BeO \cdot Al_2O_3 \cdot 6\ SiO_2$) a triclinic potassium felspar, looks so much like amazonite ($K_2O \cdot Al_2O_3 \cdot 6\ SiO_2$) that they can be distinguished only by analysis or by a microscopical determination of the crystal form. The same is true of talc ($H_2O \cdot 3\ MgO \cdot 4\ SiO_2$) and the very similar sericite ($2\ H_2O \cdot K_2O \cdot 3\ Al_2O_3 \cdot 6\ SiO_2$).

This contrasting of beryl-amazonite and talc-sericite shows that these silicates, which resemble each other so greatly, differ in that one of the pair contains potassium while the other does not. The following test can be used to distinguish these materials; it can be generally employed for establishing the presence of potassium in silicates containing at least one per cent of this element. It can be used in the field.

Chemical Basis: Decomposition of the silicate and reaction with dipicrylamine. The silicate rock is decomposed by fuming with hydrofluoric and concentrated sulfuric acids. The metals are then converted into sulfates or oxides by ignition. The potassium in the residue can be identified by the formation of red acid-resistant potassium dipicrylamine (see p. 115).

The *identification limit* of the dipicrylamine reaction is 3 γ potassium in drops.

Procedure: A small quantity (several milligrams) of the specimen is finely ground. The powder is decomposed in a platinum crucible or spoon with several drops of hydrofluoric and concentrated sulfuric acids, then carefully evaporated to dryness. Solid ammonium fluoride can be used in place of the hydrofluoric acid. When no more acid vapors are evolved, the material is ignited briefly and allowed to cool. The residue is detached from the walls of the crucible with a nickel spatula and placed on a strip of orange-red dipicrylamine paper (preparation see p. 116). After moistening with a drop of water, the paper is dried in a current of heated air and then bathed in 0.1 *N* nitric acid. The paper turns bright yellow, and if the specimen contained potassium a red fleck remains.

Suggested test materials: Potassium felspar, sodium felspar, muscovite, amazonite, beryl, talc, sericite, other silicates containing potassium, or those having little or no potassium in them.

II. Determination of Acid-Decomposable Silicates and Differentiation of Amorphous and Crystalline Silica

Silicates differ not only in the kind and content of the basic constituents (K_2O, Na_2O, MgO, etc.), but also in their resistance or non-resistance to acids, and in the state of the silica (amorphous or crystalline). Consequently, information useful in characterizing silicates can sometimes be obtained by testing their behavior toward dilute acids, and by determining whether the specimen contains crystalline or amorphous silica. These points can be simply established by the following procedures.

Chemical Basis: Behavior of silicates (silica) toward equilibrium solutions. If a neutral solution of nickel sulfate is treated with dimethylglyoxime (DH_2) the filtrate contains, in equilibrium, nickel and hydrogen ions as well as dimethylglyoxime:

$$Ni^{++} + 2\ DH_2 \rightleftharpoons Ni(DH)_2 + 2\ H^+.$$

This equilibrium is disturbed by all basic materials which consume H^+ ions, and red nickel dimethylglyoxime precipitates (see p. 225). Acid-decomposable silicates are included among such materials. Consequently, they can be detected by the production of the red nickel salt when they act on this equilibrium solution.

Another type of equilibrium solution is the filtrate obtained when silver chromate is treated with a quantity of ammonia water insufficient to dissolve the solid. This filtrate contains, in addition to free ammonia, the complex silver ammine chromate, whose ions present the following equilibrium:

$$2\ [Ag(NH_3)_2]^+ + CrO^{--} \rightarrow Ag_2CrO_4 + 4\ NH_3.$$

This yellow ammoniacal solution of $[Ag(NH_3)_2]_2CrO_4$ will precipitate brown silver chromate on contact with all materials that withdraw ammonia and therefore disturb the equilibrium (see p. 155). Amorphous silica, in contrast to the crystalline form, can bind ammonia, probably through adsorption on its extensive free surface. Consequently, it reacts with the equilibrium solution.

It is worthy of note that certain silicates react with both equilibrium solutions since they contain both acid-decomposable silicates and amorphous silica.

Procedure (detection of acid-decomposable silicates): A small quantity (1–5 milligrams) of the finely powdered specimen is placed in the depression of a spot plate and allowed to stand with one or two drops of the Ni-dimethylglyoxime equilibrium solution. The powder turns red after a few minutes if acid-decomposable silicates are present.

When using this test, care must be taken that free oxides or carbonates

are absent, since they also produce nickel dimethylglyoxime with the equilibrium solution.

The equilibrium solution is prepared as follows: 2.3 g. $NiSO_4 \cdot 7H_2O$ dissolved in 300 ml. of water is treated with 2.8 g. of dimethylglyoxime in 300 ml. of alcohol. After 30 minutes the suspension is filtered.

Suggested test materials: Garnierite; apophyllite; stilbite (natrolite) (all three are zeolites).

Procedure (detection of amorphous silica): A small quantity (1 to 5 milligrams) of a finely pulverized specimen is treated in the depression of a spot plate with one or two drops of the silver chromate equilibrium solution. Brown-red Ag_2CrO_4 separates almost immediately on the surface of the powder if amorphous silica is present.

The Ag-chromate equilibrium solution is prepared as follows: Freshly precipitated, well-washed silver chromate is added in small portions to 6 *N* ammonia until considerable quantities remain undissolved on swirling. The suspension is allowed to stand for one hour and is then filtered into a bottle that has a tightly fitting stopper. If the reagent is used repeatedly, ammonia is lost, and a slight precipitate of silver chromate appears. The solution can then be restored to usefulness by filtering it.

Suggested test materials: Garnierite, montmorillonite, kaolin, natrolite, opal.

Kieselguhr, which was long considered to be amorphous silica, has been shown by recent studies to be crystalline. It does not react with the silver chromate equilibrium solution.

8. Detection of Fluorine in Rocks and Mineral Waters

Fluorine occurs chiefly in crystalline residues of the magma; consequently many pegmatites contain considerable fluorine. The most important fluorine mineral is fluorspar or fluorite (CaF_2); cryolite (Na_3AlF_6) also is important. Apatite 3 $Ca_3(PO_4)_2 \cdot Ca(ClF)_2$, which is used for the preparation of superphosphate fertilizers, contains varying quantities of fluorine. It is known as fluor- or chlorapatite according to whether the fluorine or chlorine content predominates. The semiprecious stone topaz, $Al_2(F, OH)_2SiO_4$, contains varying amounts of fluorine.

Fluorine is present in small quantities in most soils, from which it is taken up by plants. Consequently, almost all plant ash contains small amounts of fluorine (birch leaves, for instance, about 0.1 per cent). Fluorides enter water because calcium fluoride is slightly soluble in waters containing carbon dioxide. Accordingly, many mineral waters are characterized by a slight content of fluorine.

Chemical Basis: Conversion into silicon tetrafluoride and hydrolysis to silicic acid. Both soluble and insoluble fluorides produce volatile silicon

tetrafluoride when warmed with quartz sand and concentrated sulfuric acid:

$$MeF_2 + H_2SO_4 \rightarrow MeSO_4 + 2\ HF.$$

$$SiO_2 + 4\ HF \rightarrow SiF_4 + 2\ H_2O.$$

The gas is absorbed in a drop of water and reacts to form silicic acid and hydrofluosilicic acid:

$$3\ SiF_4 + 4\ H_2O = H_4SiO_4 + 2\ H_2SiF_6.$$

Both of these products react with an acid solution of ammonium molybdate and form a yellow color due to a complex silicomolybdic acid, $H_8Si(Mo_2O_7)_6$. The molybdenum in silicomolybdic acid, as in the analogous phosphomolybdic acid, exhibits an increased reactivity toward oxidizable compounds. Like phosphomolybdic acid, the complex silicon acid can oxidize benzidine to "benzidine blue," and is itself simultaneously reduced to "molybdenum blue" (see p. 147).

This series of reactions is the basis of the following method for detecting fluorine. When applying this test, care should be taken that the decomposition with sulfuric acid does not produce other volatile compounds which can react with ammonium molybdate. The formation of hydrogen sulfide and sulfur dioxide are the only interferences of this kind likely to be encountered when testing minerals and rocks. Specimens containing sulfide should be roasted before testing for fluorine to convert the sulfides into oxide and sulfate. Ignition is also advisable with carbonates, so that the action of sulfuric acid will not liberate large quantities of carbon dioxide, whose pressure might lift the stopper of the gas evolution apparatus.

Procedure: The specimen is finely pulverized and mixed with the purest quartz sand. The mixture is placed in the gas evolution apparatus (Fig. 25, p. 51). One to three drops of concentrated sulfuric acid are added. The knob of the stopper is dipped into water and care is taken that a drop remains suspended on this projection. The apparatus is then closed carefully. It is placed on a hot plate, warmed gently for about one minute and then allowed to stand from 3 to 5 minutes. The drop on the knob of the stopper is washed into a microcrucible, 1 or 2 drops of ammonium molybdate solution are added and the mixture warmed until bubbles begin to appear. When cold, a drop of benzidine solution (1 per cent in 10 per cent acetic acid) and several drops of saturated sodium acetate solution are added. If silicic acid and consequently fluorine is present, the solution turns blue.

Mineral waters are tested for fluorine as follows: Five milliliters are placed in a small porcelain dish, a pinch (tip of knife blade) of quartz sand

added, and the suspension is evaporated to dryness. The residue is then carried through the procedure just described.

The sand used in this test is prepared by heating commercial quartz sand with concentrated sulfuric acid, washing with water and drying. The ammonium molybdate solution is prepared by dissolving 15 g. of $(NH_4)_2MoO_4$ in 300 ml. of water and then pouring this solution into 100 ml. of nitric acid (sp. gr. 1.2).

The *identification limit* of the fluorine test is 1 γ fluorine in drops.

9. Detection of Phosphate in Rocks and Minerals

Apatite 3 $Ca_3(PO_4)_2 \cdot CaCl_2$ (chlorapatite) and 3 $Ca_3(PO_4)_2 \cdot CaF_2$ (fluorapatite) is the most important phosphate rock and occurs most frequently. Apatites always are present in considerable quantities in acid granites. The phosphatic pegmatites were formed during the recrystallization of the magma. Phosphorite, $Ca_3(PO_4)_2$, is also widely distributed. The phosphate beds were produced by leaching of calcium phosphate by carbonated waters, followed by precipitation by limestone and heavy metal compounds. Extensive beds of bauxites containing phosphate are known. The bones and teeth of vertebrates contain hydroxyl apatite, 3 $Ca_3(PO_4)_2 \cdot Ca(OH)_2$.

Chemical Basis: Oxidation of benzidine by ammonium phosphomolybdate. All soluble and insoluble phosphates react with nitric acid solutions of ammonium molybdate and form a yellow crystalline precipitate, whose approximate formula is $(NH_4)_3PO_4 \cdot 12MoO_3 \cdot aq$. The molybdenum of this complex compound is more active toward many reducing agents than molybdate under normal conditions. For instance, benzidine is oxidized to benzidine blue, with simultaneous formation of molybdenum blue (see p. 146), by traces of ammonium phosphomolybdate that are too small to be seen.

The *identification limit* of this test is 0.05 γ P_2O_5 in drops.

Procedures: A granule, or several milligrams of the powdered specimen, is placed on filter paper, moistened with a drop of ammonium molybdate solution and held over a hot plate for several minutes. The paper is then spotted with benzidine solution and the moist fleck is then held over 6 *N* ammonia water. A deep blue appears on the paper beneath the specimen, or at some distance from it (sometimes in a coherent mass), wherever even traces of ammonium phosphomolybdate have formed.

The reagents are prepared as follows: Five grams of $(NH_4)_2MoO_4$ are dissolved in 100 ml. of water and the solution poured into 35 ml. of nitric acid (sp. gr. 1.2). The benzidine solution contains 0.05 g. of the base or the hydrochloride dissolved in 10 ml. of concentrated acetic acid and then diluted with water to 100 ml.

Excellent results are obtained if the test is made on a streak plate. A

streak of the specimen is spotted with the ammonium molybdate solution and allowed to stand for some time. Drops of the benzidine solution and of 6 *N* ammonia are then added in succession.

Minerals can frequently be tested *in situ*. A drop of the molybdate solution is placed on the mass and after several minutes the liquid is taken up with a piece of filter paper. The subsequent reactions are made on the paper.

This test for phosphate, with a simple modification, may be used also for the identification or detection of the localization of apatite in thin sections, or in the polished surface of a rock. The following procedure is used:

A small filter paper is soaked with the ammonium molybdate solution and the section is warmed. (In no case must the temperature be allowed to rise to the point that the Canada balsam used for imbedding becomes liquid.) If a piece of rock is being tested, it is warmed. The section, or the rock, is pressed against the moist filter paper for about 1 or 2 minutes. The filter paper, with the contact surface upward, is then laid on a strip of filter paper soaked with the benzidine solution, so that the benzidine penetrates by capillary action from below. The first paper is then held over 6 *N* ammonia and an exact picture of the apatite crystal of the section appears in blue on the filter paper. The position of the crystal in the section can thus be determined accurately. Even the smallest crystals, whose apatitic nature is doubtful, can be determined rapidly by this method, which may either replace or supplement an optical study.

Suggested test materials: Apatite, phosphorite, superphosphate.

Arsenates are quite similar to phosphates both chemically and mineralogically, but they do not, however, respond to the benzidine reaction under the foregoing conditions (see page 147). Accordingly, no confusion results and, in fact, it is possible to detect quickly the presence of phosphate in arsenates by this procedure.

10. Detection of Sulfide Minerals and Ores

Sulfidic minerals and ores, namely metal sulfides, are widely distributed as glances, blendes, and pyrites. They are very important raw materials for such commercial products as metals, sulfuric acid, etc. Consequently, the rapid recognition of the presence of a sulfidic ore is important in mineral and rock determinations, and also for technical questions concerning the working up of ores and residues.

Chemical Basis: Catalytic acceleration of the iodine-azide reaction. The reaction:

$$2\,NaN_3 + I_2 \rightarrow 2\,NaI + 3\,N_2$$

ordinarily proceeds at an immeasurably slow rate. It is catalytically accelerated by all inorganic and organic compounds that contain sulfidic

sulfur. The occurrence of the reaction can be seen easily because of the consumption of free iodine (discharge of color) and by the liberation of nitrogen (appearance of bubbles). The catalytic effect is brought about by even traces of sulfides, including those found in nature. Even though the latter are very stable toward acids, they act in this instance as rapidly as soluble or freshly precipitated sulfides. Accordingly, the test for metal sulfide can be made on the specimen itself (by applying the reagent solution to it). It is better, however, to use a finely powdered test portion, since the extensive surface brings about a quicker action than coarse pieces or larger crystal surfaces.

The iodine-azide reaction is not affected by arsenides, antimonides, tellurides, selenides, or free sulfur. However, in practice, it must be remembered that almost all minerals of these types contain small amounts of sulfide, and can, therefore, show a weak iodine-azide reaction.

Procedure I (detection by formation of nitrogen): The test can be made in various ways. The powdered specimen is covered with iodine-azide solution in a micro-test tube and the formation of small bubbles of nitrogen noted. Or a particle of the pulverized material is brought into contact with one or two drops of the reagent in an Emich centrifuge tube by means of a platinum wire as described on p. 165. It is also possible to put a drop of the reagent, by means of a pipette, on the material *in situ*, or to spot a streak of the mineral on a streak plate with the reagent. In the latter case, the streak should be as thick as possible; any coarser particles should be blown away. The reaction with the streak has the advantage that the mineral specimens are scarcely damaged.

The reagent solution consists of 1 g. of sodium azide, 1 g. of potassium iodide and a tiny crystal of iodine in 3 ml. of water. The solution can be used warm if necessary.

Procedure II (detection through consumption of iodine): A particle of the finely ground material is placed on a strip of filter paper (3 × 6 cm.) and spotted with a drop of 2 per cent sodium azide in 0.01 *N* iodine solution. A second drop of the reagent is placed on the paper at such distance from the first spot that the two do not touch. Both flecks become bright blue because of the starch in the paper. After about two minutes, the paper is immersed in water and swirled several times so that most of the mineral powder is detached. In the absence of sulfide, both flecks will be uniformly blue. If sulfide is present, a white spot surrounded by blue will be seen at the place where the sulfide could be active. Obviously, the filter paper should be tested beforehand to insure a sufficient content of starch.

Suggested test materials: Pyrites, cinnabar, galena, or other sulfide ores.

Chapter VII

TESTING OF TECHNICAL MATERIALS AND PRODUCTS WITH THE AID OF SPOT REACTIONS

The chemical testing of technical materials seldom requires complete qualitative or quantitative analyses since entirely unknown products are practically never involved. For the most part, quite definite questions are presented as to the presence or absence of certain substances which determine the excellence of the particular product or are essential in characterizing it. Frequently, information is required regarding the resistance of materials to attack by such agencies as water and air, and also how well certain chemical claims are met. Spot analysis often renders valuable services in the rapid answering of such questions; it also serves as a preliminary or orientation approach for any subsequent quantitative determinations that may be necessary.

In the first place, it may be essential to determine whether or not the claims concerning a particular product are true. In such cases, one or more of the essential constituents must be identified definitely; no particular attention is paid to the other materials that may be present. In general, this part of the testing of materials is simple. It is sufficient to dissolve small quantities of the specimen and to make characteristic identifying tests. Sometimes these can be made directly on the solid test material. Extremely sensitive tests are not necessary, since the problem is merely to identify or establish the presence of one or more of the main constituents.

Questions concerning materials which are not integral constituents arise much more frequently. They may have been added purposely or they may be present as undesirable impurities. In such investigations it must be noted whether the added materials or contaminants are distributed uniformly or whether they are localized in certain parts as inhomogeneities or segregations. The choice of the identifying reaction and the method of examination will be determined in part by these two entirely different tasks. Spot reactions will frequently serve to establish the presence or absence of the accompanying metallic or non-metallic substances determining the value of the material being tested. Important conclusions can be drawn from the results of such tests, which can be made directly on the object itself, or on solutions made from samples taken from it. Sometimes, in studies of this type, it is well to use different tests with quite distinct sensitivities, because the positive or negative responses then give a measure of information as to the quantities of the pertinent material(s) present.

In many instances the accompanying materials are tolerated, provided their quantity does not exceed certain limits; the proof of their presence

then serves especially as a check on constancy of quality or origin. High purity is desirable or demanded in some cases because the degree of purity determines the usefulness and therefore the value of the product. A special division of the chemical testing of materials, namely tests of purity, deals with investigations having this in view. The purity of a product, as a rule, is established by convention, because it is impossible to set up a specification of purity which will be valid in all cases. Aside from the fact that absolute purity is found in very few materials, chemical testing of purity is dependent, above all, on the sensitivity and certainty of the methods employed. These vary within wide limits. For example, it is possible to detect traces, in the real sense of the word, of arsenic, copper, iron, and other elements in materials. In contrast, the detection of potassium in sodium salts (and vice versa) is possible, only if the foreign element is present in amounts exceeding a relatively quite high percentage, because very sensitive and sure methods of detecting potassium (or sodium) are not available. A search for traces can be of great importance when testing the purity of materials, because even extremely minute quantities of the foreign substance sometimes greatly affect the chemical and physical properties, particularly those of metals. Tasks of this kind naturally demand the use of the most sensitive tests and these can sometimes be accomplished excellently in the form of spot reactions. In such instances also it is possible, at times, to make the test directly on the specimen being examined. Usually, however, a special preparation or accumulative procedure is required as a preliminary to the spot reaction.

Many technical materials are required to meet certain demands without detectable damage. Consequently, the determination of the resistance toward chemical and physical actions (water, acids, alkalies, chemicals, increased temperatures and pressures) are the subject of special tests, whose results are often of decisive significance in determining the use to which the material can be put. As a rule, tests of this kind are carried out by exposing the material (either in masses or as a powder) to a chemical or physical agency and then determining whether certain constituents have dissolved or been otherwise altered. Valuable clues as to the extent of the resistance can sometimes be obtained from drops of the solution tested by spot reactions with different sensitivities.

The foregoing comments have not completely covered the great field of the chemical testing of materials. The determination and the cause of the changes undergone by such materials when stored, transported, and during processing, are of great importance. It is often necessary to keep in mind that only small quantities can be available for studies of this kind, so that here again the problem of searching for traces may become of practical importance. The same is true of examinations required for legal purposes

in criminal investigations. In these, the preservation of the results of the test in the form of permanent preparations may have high documentary value.

1. Detection of Lead in Alloys, Pigments, Glass, etc.

The definite and rapid detection of lead is often desirable in examining various materials and establishing purity. Certain bronzes, for instance, differ in their lead content; some inorganic earth colors (pigments) may be characterized by their lead content; the presence or absence of lead plays a rôle in determining the value of some glasses. Testing for lead is also important in examinations of foods.

The following procedure permits the rapid and definite recognition of lead, without requiring the tedious preparation of a special solution of the test portion.

Chemical Basis: Formation of lead rhodizonate. Pb^{++} ions react, in a solution whose pH is approximately 3, with sodium rhodizonate to form a red precipitate. The reaction is so sensitive that the buffer solution used to regulate the pH may also be used to dissolve lead from alloys, etc. Under these conditions no other metals interfere.

The *identification limit* of this test is 0.1 γ lead in drops.

Procedure: A little of the powdered specimen (a few filings of alloys, etc.) is stirred in the depression of a spot plate with 3 drops of the buffer solution. After about 2 minutes, a drop of sodium rhodizonate solution is added. When lead is present, a red precipitate or color appears, depending on the quantity. With small amounts of lead it is best to make a blank test with the buffer and the sodium rhodizonate solution; the mixture becomes almost colorless after about 2 minutes.

The reagent is a 0.2 per cent solution of sodium rhodizonate. It must be freshly prepared; it can be kept in the refrigerator about 3 days. The buffer solution (pH = 2.87) contains 15 g. tartaric acid and 19 g. sodium bitartrate per liter.

Suggested test materials: alloys (brass, bronzes, etc.), chrome yellow, Naples green, lead glass, driers, insecticides (lead arsenate).

See p. 134 concerning the detection of traces of lead in metal salts.

2. Detection of Traces of Cadmium in Copper or Zinc

Cadmium should always be looked for when testing the purity of metallic copper or zinc. It is particularly apt to be present, in varying quantities, in crude copper or zinc, and traces may even find their way into the refined metals. The detection of small quantities of cadmium in these two metals is difficult and tedious when the classical analytical methods are used, because the chemical reactions of cadmium are quite similar to those of

copper and zinc. The method described here permits the rapid and definite detection of traces of cadmium in copper or zinc. Appropriately modified, it can also be used to test for cadmium in other metals.

Chemical Basis: Precipitation of ferrous α,α′-dipyridyl-cadmium iodide. Ammoniacal solutions of cadmium salts contain $Cd(NH_3)_4^{++}$ ions. On the addition of $Fe(\alpha,\alpha'\text{-dip})_3^{++}$ ions and iodide ions, a red crystalline precipitate separates immediately:

$$Cd(NH_3)_4^{++} + Fe(\alpha,\alpha'\text{-dip})_3^{++} + 4\ I^- = Fe(\alpha,\alpha'\text{-dip})_3CdI_4 + 4\ NH_3.$$

The $Fe(\alpha,\alpha'\text{-dip})_3^{++}$ ions are obtained by treating a solution of a ferrous salt with α,α′-dipyridyl hydrochloride.

The *sensitivity* of the test for cadmium (see p. 121) is 0.05 γ Cd in drops.

Since copper and zinc salts, in ammoniacal solution, do not react with Fe(II)α,α′-dipyridyl and iodide ions, and do not interfere with the precipitation of the cadmium salt, cadmium can be detected directly in the presence of very large quantities of copper or zinc. If cadmium is present along with metal ions which produce a precipitate with ammonia, the precipitate is filtered off and the test for cadmium made on the filtrate.

It must be remembered that a mixture of $Fe(\alpha,\alpha'\text{-dip})_3^{++}$ ions and I^- ions is not stable at all concentrations; a black-red precipitate of crystalline $[Fe(\alpha,\alpha'\text{-dip})_3]I_2$ forms in concentrated solutions. Consequently, a solution which still contains potassium iodide must be used as the precipitant. A solution of this kind contains all the ionic species necessary to precipitate the cadmium ions in accord with the foregoing equation.

Procedure: The reagent solution is prepared as follows: 0.25 g. of α,α′-dipyridyl and 0.146 g. $FeSO_4 \cdot 7H_2O$ are dissolved in 50 ml. water and then 10 g. KI added. After thorough shaking, the precipitate is filtered off. The solution keeps well. If it becomes turbid, it can be restored to usefulness by filtering.

One to two grams of the metal to be tested for cadmium is dissolved in nitric acid in a porcelain crucible. The solution is evaporated to dryness and the residue is taken up in 1 ml. of ammonia water. Filtration or centrifuging is necessary only if a considerable precipitate is produced. One drop of the ammoniacal solution is placed on filter paper (S. and S., 601 or Whatman, 120) and, before it sinks in, is treated with one drop of the reagent solution. A red fleck or ring appears when cadmium is present. A positive result is easily seen, because the fleck stands out plainly against the reagent left in the paper.

This procedure *permits the detection*, in one drop of

0.08 γ cadmium	in the presence of 500 times	copper
0.1 γ cadmium	this quantity of	zinc

Suggested test materials: crude zinc, crude copper; zinc dross.

3. Detection of Nickel in Electroplatings and Alloys

Since nickel resists the action of air and moisture, many tools, utensils, etc. are made of nickel or are electroplated with nickel. This metal is often added to alloys because it disguises the color of the copper. A rapid test for nickel is therefore desirable in many cases. The present procedure is electrographic; it can be accomplished without visible damage to the specimen.

Chemical Basis: Formation of red nickel dimethylglyoxime following anodic solution of metallic nickel. Nickel ions produce a red precipitate with dimethylglyoxime. Sufficient Ni^{++} ions can be obtained without dissolving the entire sample, if the article or material to be tested is made the anode in a circuit, whose cathode is a strip of aluminum foil. Moist filter paper impregnated with dimethylglyoxime is placed between the electrodes. The current is furnished by a flash light battery. When the circuit is closed, the nickel dissolves at the anode and migrates toward the cathode. On the way, however, it is precipitated by the dimethylglyoxime, and a red fleck appears on the white paper where the specimen makes contact. Under these conditions copper or iron are also anodically dissolved. Cupric and ferrous solutions form brown or red salts respectively with dimethylglyoxime. However, these products, in contrast to nickel dimethylglyoxime, are soluble in water and accordingly can be washed out of the paper.

Procedure: The reagent paper is prepared by bathing strips of filter paper in a 1 per cent alcohol solution of dimethylglyoxime. After draining, the paper is dried with warm air. A strip of this impregnated paper is moistened with water and laid on the aluminum foil that serves as cathode of the electrospot apparatus shown in Fig. 39. The object to be tested (foil, wire, chips, etc.) is placed on the reagent paper and the circuit is closed by pressing the copper plate which serves as anode. After about a minute the current is interrupted and the anode and the test piece are removed. If nickel is present, a red fleck, which does not disappear on washing, is left on the white paper.

Suggested test materials: Alloys containing nickel.

4. Detection of Traces of Nickel in Cobalt Salts

Since cobalt and nickel always occur together in ores and minerals, cobalt salts derived from these sources practically always contain varying quantities of nickel even after industrial separations and purifying processes. It is not feasible to test cobalt salts directly for nickel by means of the sensitive dimethylglyoxime reaction. Cobaltous ions produce a brown color due to soluble complex compound containing di- and trivalent cobalt. This reaction consumes dimethylglyoxime, whose solubility is

limited; consequently not enough of the reagent is left to reveal minute quantities of nickel. Furthermore, nickel dimethylglyoxime is quite soluble in concentrated solutions of cobalt dimethylglyoxime salts. For this reason, neither the detection nor the determination of nickel in cobalt salts was previously possible for a quantity of about 0.03 mg. nickel, when the ratio of Ni:Co was greater than 1:100.

The following tests will reveal traces of nickel in cobalt salts and, with appropriate modifications, can also be used to prepare cobalt salts which are practically free of nickel.

Chemical Basis: Precipitation of nickel dimethylglyoxime after masking the cobalt as $Co(CN)_6^{---}$ ion. Nickel and cobalt salts are first precipitated as cyanides, which are soluble in an excess of the alkali cyanide. The complex $K_4Co(CN)_6$ and $K_2Ni(CN)_4$ result. These complex cyanides do not react with dimethylglyoxime because the solutions contain too little Ni^{++} or Co^{++} ions to exceed the solubility or the ion product of the respective dimethylglyoxime salts. Hydrogen peroxide converts the complex cobaltocyanide into soluble complex cobalticyanide, $K_3Co(CN)_6$, which is completely resistant to dimethylglyoxime and also to formaldehyde. In contrast, $K_2Ni(CN)_4$ is not affected by hydrogen peroxide, but formaldehyde converts it into $Ni(CN)_2$, which reacts promptly with dimethylglyoxime. Consequently, the cobalt can be masked completely by producing the complex cyanide and then oxidizing it. The nickel can then be precipitated with dimethylglyoxime after the complex nickel cyanide has been decomposed with formaldehyde.

The reactions underlying the detection of traces of nickel in cobalt salts are:

(I) Conversion of Cobalt and Nickel into soluble complex cyanides.

$$Co^{++} + 2\,CN^- \rightarrow Co(CN)_2 \qquad Ni^{++} + 2\,CN^- \rightarrow Ni(CN)_2$$

$$Co(CN)_2 + 4\,KCN \rightarrow K_4Co(CN)_6 \qquad Ni(CN)_2 + 2\,KCN \rightarrow K_2Ni(CN)_4$$

$$2\,K_4Co(CN)_6 + H_2O_2 \rightarrow 2\,K_3Co(CN)_6 + 2\,KOH$$

(II) Decomposition of the complex nickel salt by formaldehyde:

$$K_2Ni(CN)_4 + 2\,CH_2O \rightarrow Ni(CN)_2 + 2\,CH_2\begin{cases}OK\\CN\end{cases}$$

(K salt of glycolic nitrile)

(III) Precipitation of nickel dimethylglyoxime:

$$Ni(CN)_2 + 2\,C_4H_8N_2O_2 \rightarrow Ni(C_4H_7N_2O_2)_2 + 2\,HCN.$$

The following procedure will reveal the presence of nickel even in high-grade cobalt salts, such as "for analysis", formerly regarded as nickel-free.

It is advisable to run a parallel test with an absolutely nickel-free cobalt salt, whose preparation is described presently.

Procedure: A portion of the soluble cobalt salt to be tested, the size of a pea (about 0.05 g.), and a like quantity of the nickel-free cobalt salt are placed in adjacent depressions of a spot plate. The samples are dissolved in 1 or 2 drops of water and then drops of saturated potassium cyanide solution are stirred in until the precipitate first formed has redissolved. One or two drops of 3 per cent hydrogen peroxide are then added to oxidize the cobaltocyanide to cobalticyanide. The mixture is stirred occasionally until the solution becomes bright yellow (color of the $Co(CN)_6^{---}$ ions). This requires several minutes. A pinch (tip of knife blade) of solid dimethylglyoxime is added and then several drops of 40 per cent formaldehyde. The mixture is stirred. When nickel is present, the solution turns orange-red, or the red nickel dimethylglyoxime precipitates. The parallel test conducted on the nickel-free preparation remains unchanged (yellow). Very minute color differences, produced by traces of nickel can be distinguished by comparison with the nickel-free preparation.

Suggested test materials: Technical cobalt sulfate and "nickel-free" cobalt salts.

Preparation of Cobalt Salts Quite Free of Nickel

Ten grams of a "nickel-free" cobalt salt are dissolved in water, the solution warmed and treated with a concentrated solution of potassium cyanide until the precipitate, formed at first, dissolves. A clear green-yellow solution results. The cobalt is then converted into potassium cobalticyanide, $K_3Co(CN)_6$, by adding 3 per cent hydrogen peroxide and boiling for a few minutes. The liquid should become honey-yellow; if not, more peroxide must be added. Any slight precipitate is removed by filtering; then the solution is evaporated to a syrupy consistency. An excess of solid dimethylglyoxime is added and the lukewarm solution is stirred well with 40 per cent formaldehyde. On cooling, nickel dimethylglyoxime, and solid dimethylglyoxime precipitate. The suspension is allowed to stand for an hour and then filtered. The filtrate, which is now free of nickel, is taken to dryness on a sand bath. The residue is carefully heated, with constant stirring, until the mass begins to char and appears quite black. After cooling slightly, the residue is stirred to a paste with warm water. Concentrated hydrochloric acid is added and the mixture warmed on the water bath for 1 to 2 hours. The suspension is then diluted and filtered. The filtrate is set aside temporarily. The residue is dried, ignited, stirred to a paste with water, treated with concentrated hydrochloric acid, diluted and filtered. The two filtrates are then united. Caustic alkali is added; this precipitates cobaltous hydroxide, which is filtered, washed thoroughly

and then ignited to the oxide. It can be converted into metallic cobalt by reduction in hydrogen. The washed oxide or the metal can be dissolved in acids; the nickel-free cobalt salts are obtained by crystallization. These can be used for the parallel tests required as specified in the preceding section.

5. Detection of Traces of Metallic Zinc in Zinc Oxide

Detection of Free Metals

Pure zinc oxide can be prepared by wet methods (e.g., by ignition of precipitated zinc carbonate) or by burning zinc vapor in air. The zinc oxide produced from solutions is used for pharmaceutical purposes (various zinc preparations). As a rule, it is denser then the very fine oxide obtained by burning zinc. The latter product has a high covering power and therefore is used as a pigment by artists and in cosmetics.

The detection of metallic zinc can be used to distinguish the two varieties of zinc oxide and also to evaluate zinc oxide. When produced by dry methods, zinc oxide often contains traces of the metal which are not visible because they are coated with the oxide. Nevertheless, this inclusion diminishes the covering power of zinc oxide.

Chemical Basis: Solution of metals by phosphomolybdic acid with production of "molybdenum blue." Phosphomolybdic acid, $H_3PO_4 \cdot 12MoO_3 \cdot 6H_2O$, is an acid of medium strength which may also function as an oxidizing agent. Consequently, phosphomolybdic acid dissolves not only those metals above hydrogen in the electromotive series but also more noble metals (Cu, Ni, Co, Ag, Hg) which do not dissolve in dilute hydrochloric or sulfuric acid. The solution of metals by the action of phosphomolybdic acid on compact pieces as well as on powders can be directly revealed by the formation of "molybdenum blue." The latter is produced by the partial reduction of the sexivalent molybdenum to the quinquevalent stage of oxidation.

Procedure: Portions (10 mg.) of zinc oxide are placed in four adjacent depressions of a spot plate. *Without stirring*, 3 drops of a saturated aqueous solution of phosphomolybdic acid and one drop of 6 *N* sulfuric acid are added to each. The zinc oxide dissolves slowly; if traces of metallic zinc are present, blue dots (molybdenum blue) appear at the site of the metal. The dots are quite visible against the white background of the spot plate and the still undissolved zinc oxide. On standing, the blue points disappear because the soluble molybdenum blue diffuses away.

If two kinds of zinc oxide are to be compared with respect to the content of metallic zinc, equal quantities of the specimens should be tested by the foregoing procedure in two neighboring rows of depressions of the spot plate.

Blue dots never appear if the zinc oxide contains no free metal. The number of blue dots is proportional to the content of metallic zinc in the oxide.

Zinc oxide "for analysis" (Merck and also Kahlbaum-Schering) gave a positive response to this test for metallic zinc. On the other hand, zinc oxide "C.P." furnished by Coleman and Bell was found to be entirely free of the metal.

Litharge (PbO) prepared in the dry way by oxidation of molten lead, as well as red lead (Pb_3O_4) obtained by oxidation of PbO, always contain some metallic lead. The test for free lead can be made in the same way as the test for zinc in zinc oxide, if nitric acid is used in place of sulfuric acid as the solvent. Lead oxide produced by ignition of $Pb(OH)_2$ or $Pb(NO_3)_2$ contains no metallic lead.

If metal powders (printing on paper, wrappers, etc.) are to be detected it suffices to spot a small strip of the specimen with a saturated solution of phosphomolybdic acid and to look for any blue coloration.

Suggested test materials: Zinc oxide from various sources, litharge, red lead, granulated metals (Mg, Al, Cu, Co, Ag, etc.), wrappings and printing on cigarette packages.

6. Detection of Traces of Iron

In Fluorides, Mercury Salts, et cetera

Traces of iron should always be looked for when testing the purity of chemicals. The iron comes from the raw materials and the reagents used during the course of the preparation, from the equipment, from dust, from the packing materials, etc. and, as they have not been fully eliminated, traces enter the finished product.

The usual identifying reactions for ferric iron with alkali thiocyanate or ferrocyanide cannot be used in certain cases. For instance, these reactions fail when they are applied in solutions containing fluorides or phosphoric acid. The iron is no longer present as Fe^{+++} ions, but as complex FeF_6^{---} or $Fe(PO_4)_2^{---}$ ions which do not produce a red solution with thiocyanate or a blue precipitate with ferrocyanide. In short, the iron is masked.

Likewise slight quantities of iron in mercury salts cannot be detected by means of thiocyanate or ferrocyanide, because Hg^{++} ions with thiocyanates form complex, colorless $Hg(CNS)_4^{--}$ ions. The Prussian blue reaction is indeterminate because of the concurrent production of yellow insoluble mercuric ferrocyanide.

Consequently, in all cases where masking of ferric ions may be involved, or where thiocyanate or ferrocyanide ions are consumed by extraneous reactions, it is advisable to test for iron by the following procedure which is also suitable for the identification of iron in insoluble compounds.

Chemical Basis: Conversion of iron into Fe^{++} ions and detection with

α,α′-dipyridyl (*α,α′-phenanthroline*). All soluble iron salts are reduced immediately to the ferrous condition by hydrogen sulfide or sulfurous acid and can then be detected by the sensitive α,α′-dipyridyl or α,α′-phenanthroline reactions. Deep red $Fe(\alpha,\alpha'\text{-dip})_3^{++}$ or $Fe(\alpha,\alpha'\text{-phen})_3^{++}$ ions are formed (see p. 119). As the reaction occurs in acid, neutral, or ammoniacal solution, insoluble ferric compounds can be simultaneously dissolved and reduced with hydrochloric acid solution of stannous chloride and then subjected to the test.

The *sensitivity* of the test for iron with α,α′-dipyridyl or α,α′-phenanthroline is 0.03 γ Fe in drops.

Procedures:

1. *Testing of fluorides:* One milliliter of the solution to be tested is placed in a paraffined porcelain microcrucible and treated with one drop of colorless ammonium sulfide solution. After one minute a drop of a 2 per cent solution of α,α′-dipyridyl (or α,α′-phenanthroline) in dilute hydrochloric acid is added. In every case, additional dilute hydrochloric acid should be introduced to assure acidity of the mixture. A red to pink color appears depending on the iron content.

The crucible must be paraffined to prevent the hydrofluoric acid liberated on the addition of the acid reagent solution from dissolving traces of iron from the walls of the crucible. The coating is best applied by melting a piece of paraffin the size of a pea in the crucible and then swirling so that the liquid paraffin will cover the walls when it solidifies.

Identification Limit: 4 γ Fe in the presence of 1 g. K_2F_2.

2. *Testing of mercury salts:* One or two drops of the solution to be tested is treated in a test tube with an equal volume of the reagent solution. Some solid sodium sulfite and sodium chloride are added and the mixture warmed. When traces of iron are present, a pink color develops at once.

The sodium chloride is added to form Na_2HgCl_4. This prevents the precipitation of a white insoluble double salt of $HgCl_2$ with α,α′-dipyridyl (phenanthroline).

3. *Testing of alumina or pyrolusite:* A piece of alumina the size of a pea is ground to a fine powder and decomposed by fusion with potassium bisulfate in a porcelain crucible. The melt is dissolved in water, and sodium sulfite together with 1 or 2 drops of the acid solution of α,α′-dipyridyl (phenanthroline) introduced. A pink coloration appears when the iron content is small.

Pryolusite is tested for iron as follows: Several milligrams of the finely ground sample are mixed in a microcrucible with 1 or 2 drops of a weakly acid solution of stannous chloride. The mixture is warmed gently and then cooled. A red to pink coloration, depending on the iron content, appears when the reagent is added.

Stannous chloride sometimes contains a slight quantity of iron. Consequently, it should be tested beforehand for iron. In all cases (if the iron content of the stannous chloride is small) a parallel test should be made.

Suggested test materials: Technical potassium fluoride, technical mercuric chloride, alumina containing iron, such as white bauxite; naturally occurring pyrolusite, or manganese dioxide taken from a flash light battery.

7. Detection of Inorganic and Organic Compounds Which React with Mineral Acids

The identification of rocks and minerals and the chemical examination, for various purposes, of technical materials often require a rapid method of detecting such water-soluble or insoluble products as react with dilute mineral acids to form the corresponding salts. Compounds of this type, which in the widest sense can be regarded as "basic", include hydroxides, oxides, carbonates, arsenates, phosphates, fluorides, organic bases, and salts of weak organic acids.

Instances in which the following method of detecting basic compounds and materials attacked by acids can be applied, are:

1. The recognition of rocks susceptible to attack by acids (oxides, carbonates, phosphates, acid-decomposable silicates, etc.).
2. Detection of acid-soluble metals and alloys.
3. Testing of evaporation and ignition residues.
4. Detection of potassium chromate in potassium bichromate.
5. Detection of free bases and salts of weak acids in organic mixtures.

Chemical Basis: Precipitation of nickel dimethylglyoxime from a Ni^{++}-dimethylglyoxime equilibrium solution. The red nickel salt is only partially precipitated if dimethylglyoxime is added to a solution of the nickel salt of a strong acid (chloride, nitrate, or sulfate). If the precipitate is removed, the filtrate is a solution saturated with nickel dimethylglyoxime and presents the equilibrium:

$$Ni^{++} + 2\ DH_2 \rightleftharpoons Ni(DH)_2 + 2\ H^+.$$

The pH of this type of equilibrium solution is 1.9. The solution reacts with all materials that consume H^+ ions. The equilibrium is thus disturbed leading to a deposition of red nickel dimethylglyoxime, which can be seen easily even in minute amounts.

Another less stable but still more sensitive equilibrium solution can be prepared by treating nickel sulfate solution with an alcoholic solution of thionalid (thioglycolic-β-aminonaphthalide) and filtering.

The advantage of using such equilibrium solutions for the detection of basic compounds is that it is easily possible to discover rapidly even materials so slightly soluble in water that they do not react with the usual indicators. The test succeeds with quite minute quantities of solids.

Procedure: The nickel-dimethylglyoxime equilibrium solution is prepared as follows: 2.3 g. $NiSO_4 \cdot 7H_2O$ dissolved in 300 ml. of water is treated with 2.8 g. dimethylglyoxime dissolved in 300 ml. of alcohol. After 30 minutes, the suspension is filtered. The filtrate will keep for several weeks in glass bottles. Several grains of the finely powdered sample are placed on a white porcelain spot plate. One or two drops of the equilibrium solution are mixed with the specimen with a fine glass rod or by blowing through a glass capillary. Depending upon the susceptibility of the sample to the acid contained in the equilibrium solution, there is either no change, or a red color appears immediately or after several minutes. Very small quantities can be tested on a slide and the color observed under the microscope. Specimens of rocks and minerals can be scraped with a pocket knife and the powder then spotted directly on the solid specimen itself.

Only in the case of colorless materials can the production of the red precipitate be detected immediately. If colored products are being tested it is best to make blank tests, substituting several drops of water for the equilibrium solution.

The analyst is urged to try the test with basic compounds such as $MgNH_4PO_4$, albumen, etc., which have no effect on indicator solutions (phenolphthalein). They react promptly with the equilibrium solution.

Aluminum oxide reacts with the equilibrium solution. This finding can be used to identify alumina. An aluminate solution is obtained in the usual scheme of qualitative analysis; hydrated alumina is precipitated from this by ammonium chloride or by acidifying and then adding ammonium hydroxide. If a gelatinous precipitate forms, it is considered as proof of the presence of aluminum in the original sample. It frequently happens, however, that even in the absence of aluminum, a gelatinous precipitate resembling hydrous alumina is obtained by this procedure. This precipitate is silica, extracted from the glass vessels by strong caustic alkalies. Consequently, if in a qualitative analysis, a slight precipitate is obtained, it is always necessary to verify the identification of aluminum. Formerly this was not easy; consequently the erroneous reporting of aluminum was one of the most frequent mistakes in inorganic qualitative analyses. The identification can be made with the aid of the equilibrium solution. The precipitate, presumably hydrous alumina, is carefully washed free of alkali, that is, until the filtrate no longer responds positively when tested with the equilibrium solution. The solid is then either spotted on the filter, with the equilibrium solution, or portions are tested on a spot plate. A red that appears immediately and becomes increasingly more intense on standing proves the presence of aluminum oxide.

Suggested test materials: Hydrous aluminium oxide, aluminum oxide, calcium carbonate, magnesium ammonium phosphate, metallic magnesium, magnesite, lead carbonate, metallic zinc, zinc carbonate, bentonite, bauxite,

kaolin, pumice, kieselguhr (not ignited), potassium chromate and bichromate (both in solution), fusible and infusible white precipitate ($HgNH_2Cl$ and $Hg(NH_3)_2Cl_2$), benzidine base, hexamethylenetetramine, α-naphthylamine.

8. Detection of Alkali in Ash, Evaporation Residues of Water, and so forth

Varieties of paper that are apparently alike can often be differentiated by testing the ash for fixed alkali. For instance, qualitative and quantitative filter papers often look very much alike but the ashes differ markedly with respect to quantity and alkali content. The same is true of the ash of various natural and artificial charcoals. Such differences are easily determined by means of spot reactions and small quantities of the specimens suffice for these tests.

Chemical Basis: Test for fixed alkali by reaction with silver-manganese nitrate. All compounds that furnish hydroxyl ions react immediately with a solution containing Mn^{++} and Ag^+ ions. A solution of the nitrates is best. The reaction:

$$4\ OH^- + 2\ Ag^+ + Mn^{++} \rightarrow 2\ Ag + MnO_2 + 2\ H_2O$$

produces a deep black adsorption complex of metallic silver and manganese dioxide. Consequently, if the ash of paper or charcoal, or the gently ignited residues from water, be moistened with the reagent solution, a gray to black stain appears, according to their content of fixed alkalies, CaO, etc.

The *identification limit* of the test for alkali with silver-manganese reagent is 0.1 γ K_2O.

Procedure: The reagent solution contains 2.87 g. $Mn(NO_3)_2$ and 3.39 g. $AgNO_3$ dissolved in 100 ml. of water and then treated drop-wise with dilute alkali until precipitation begins. The filtrate is used. To test the ash of paper, a small strip (approximately 2 × 3 mm.) is incinerated on a porcelain crucible lid until the ash becomes as white as possible. After cooling, it is spotted with a drop of the reagent solution. The ash immediately, or in a few minutes, turns gray to black, depending on the alkali content. The ash of cigarette-, writing- or newspaper turns dark black immediately, indicating a high alkali content. The same is true of qualitative filter paper, whereas the ash of quantitative paper hardly reacts.

The alkali content of a charcoal is tested by ashing about 0.1 g. of the sample on a porcelain crucible lid and spotting the cooled residue with a drop of the reagent.

To differentiate hard from soft water, or to determine whether the sample is distilled water, a drop is evaporated on a porcelain crucible lid, the residue ignited, cooled, and spotted with the reagent. The intensity of the black or gray, which, if necessary, can be compared with standards carried

through the same procedure, establishes the nature of the sample. The finished colored test specimens can be preserved.

Suggested test materials: Qualitative and quantitative filter paper, writing paper, charcoal, coal, bone black, activated charcoal, distilled and well water.

9. Detection of Traces of Hydrogen Sulfide in Water and Traces of Sulfur in Organic Solvents and Fuels

Palatable drinking water must contain no hydrogen sulfide. Water to be used for technical purposes (service or tap water) may contain no more than traces of H_2S. Consequently, every chemical examination of water must include a test for sulfuretted hydrogen.

The test for free sulfur in organic solvents and fuels is often important because sulfur can lead to serious corrosion of metals in contact with the organic liquids. This test for hydrogen sulfide can be applied, in the same form, for the detection of dissolved free sulfur.

Chemical Basis: Formation of mercuric sulfide and its detection by catalytic acceleration of the iodine-azide reaction. Free mercury has so great an affinity for sulfur that, if water containing hydrogen sulfide or an organic liquid containing dissolved sulfur, is shaken with a drop of mercury, mercuric sulfide is formed on the surface. This can be detected by bringing the sulfided mercury drop in contact with an iodine-azide solution. The test will reveal invisible minute quantities of mercuric sulfide produced from slight quantities of H_2S or S. The mercuric sulfide catalyzes the immeasurably slow reaction:

$$2\,NaN_3 + I_2 \rightarrow 2\,NaI + 3\,N_2.$$

The formation of small bubbles of nitrogen will be observed (see p. 165).

Procedure: The iodine-sodium azide solution consists of 3 g. sodium azide dissolved in 100 ml. of 0.1 *N* iodine. Ten milliliters of the water to be tested is shaken vigorously for several minutes in a hard glass test tube or a stoppered measuring cylinder with a drop of mercury taken from a mercury dropper (see p. 36, Fig. 10). The water is poured off and the mercury transferred to a watch glass. The rest of the water is removed by filter paper applied to the side of the drop. One or two drops of iodine-sodium azide solution are added. Some of the bubbles of nitrogen will stick on the surface of the mercury and are easily seen under a magnifying glass.

When testing for sulfur in organic liquids, the sample is shaken with mercury and then *completely volatilized* before applying the test.

Identification Limit: 0.05 γ H_2S in 10 ml.

Concentration Limit: 1:200,000,000.

Still smaller H_2S contents can be detected if a larger volume of the water is shaken in successive 10 ml. portions with a drop of mercury. This is then tested by the iodine-azide reaction.

10. Detection of Sulfur That Can Be Extracted from Ores and Technical Materials

It is often desirable, when examining technical materials, to be able to detect rapidly the presence of any free sulfur that can be extracted with carbon disulfide. For instance, numerous sulfide ores (polysulfides, pyrites, marcasites, many galenas) contain such sulfur as an original ingredient. Sometimes part of the sulfur of these ores is liberated as a result of oxidation when they are stored or weathered. A coprecipitation of sulfur, whose presence or removal must be checked, often occurs during sulfide precipitations made in the course of analytical procedures. Free sulfur is an ingredient of many fungicides, particularly those used in vineyards. Pharmaceutical preparations may contain sulfur, either as an active ingredient or as an undesirable decomposition product. The detection of free sulfur is important when examining vulcanized rubber; the free sulfur content should be low.

When extracting sulfur it should be remembered that the best extraction medium, carbon disulfide, dissolves only the crystalline modifications of sulfur. It does not dissolve amorphous sulfur which may be formed when sulfur-bearing compounds decompose. Hot pyridine will extract *all* modifications of sulfur; however its solvent power for this element is far below that of carbon disulfide.

Chemical Basis: Addition of sulfur to thallium sulfide. Sulfur, dissolved in an organic solvent, forms an addition compound with thallium sulfide precipitated in the capillaries of paper and therefore finely divided. The resultant product differs from the black thallium sulfide in color (red-brown) and in its resistance toward dilute acids (see p. 96). Considerable quantities of sulfur can be detected directly by the formation of red-brown flecks after evaporation of the solvent. Small quantities react on the surface of the thallium sulfide distributed in the paper and protect the underlying thallium sulfide against the action of acids (see "Protective Layer Effect," p. 85). The thallium sulfide reagent paper should be rather freshly prepared, since its stability is limited (about 4 days). The preparation of this reagent paper is described on p. 85.

The *identification limit* on thallium sulfide paper is 3 γ sulfur in drops.

Procedure I (orientation test): A small quantity (1 or 2 mg.) of the dry pulverized specimen is placed on thallium sulfide paper and spotted with a drop of carbon disulfide. The solvent is evaporated (blast of heated air) and the spotting with drops of carbon disulfide is repeated once or twice more. The solid is then removed from the paper by means of a brush and

the paper then bathed in 0.5 *N* nitric acid. A red-brown fleck will be left if free sulfur is present.

Procedure II: The test portion is ground as finely as possible and extracted with carbon disulfide or pyridine in a micro-extraction apparatus. A drop of the extract is placed on thallium sulfide paper and dried in a blast of heated air. It should be noted whether the spot turns brown, an indication of the presence of about not less than 30 γ sulfur in drops. The reagent paper is then placed in 0.5 *N* nitric acid. The thallium sulfide dissolves; a brown fleck remains on the spotted area only if sulfur was present.

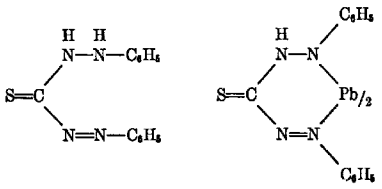
Free sulfur in vulcanized rubber can be detected by shaking a little of the sample, cut into pieces, with 2–3 ml. of carbon disulfide in a test tube. After about 3 minutes, a drop of the solution is placed on thallium sulfide paper and treated by the procedure just described.

Suggested test materials: Pyrites, rubber hose, rubber stoppers, purification masses from gas works.

11. Detection of Tetraethyl (-phenyl) Lead in Motor Fuels

Certain metallo-organic compounds (metal alkyls, -aryls, -carbonyls, and acetylides) are added to mineral oils because, even in small amounts, they retard certain undesirable reactions, chiefly by lowering the reaction rate. Metallo-organic compounds particularly, are used as anti-knocks and as anti-oxidants in hydrocarbon oils. Since the widely used tetraethyl (-phenyl) lead is extremely volatile, and has a very toxic effect on the nervous system, a sure test for this compound is at times of the greatest importance. The procedure described here is applied easily to gasolines and other motor fuels.

Chemical Basis: Photochemical decomposition of tetraethyl (-phenyl) lead and detection of lead with dithizone. Ultraviolet light almost immediately decomposes finely divided $Pb(C_2H_5)_4$ and $Pb(C_6H_5)_4$. Butane (or diphenyl) and free lead are probably formed. The latter is partially oxidized. Metallic lead and also all lead salts undergo a very sensitive reaction with diphenylthiocarbazone (I), commonly known as dithizon. A deep red inner complex salt of lead is formed (II).



(I)

Dithizon reacts with numerous other metals, usually forming red inner complex salts. In the present case, however, the test for lead in motor fuels is unequivocal.

The *sensitivity* of the dithizon reaction is 0.04 γ Pb in drops.

Procedure: One or two drops of the gasoline, etc., are placed on filter paper and irradiated under the quartz lamp until completely evaporated. The spot is then treated with a freshly prepared solution (0.1 per cent) of dithizon in chloroform. When tetraethyl (-phenyl) lead is present, the site of the gasoline fleck becomes deep red; otherwise in the absence of lead salts the fleck turns green. It is necessary to shake highly colored gasolines with activated charcoal before proceeding with the test.

Oils usually have an intense self-color. If they are to be tested for lead, 100 to 250 ml. of the sample are mixed with 3 per cent of ordinary benzene (C_6H_6), and then steam distilled. The distillate is tested for lead by the procedure just given.

An alternate procedure is to place a drop of the leaded fuel on the surface of a 1 per cent potassium cyanide solution in a microcrucible. The specimen is then irradiated for about 30 seconds with ultraviolet light. A drop of dithizon solution will produce a red coloration if lead is present.

Traces of nickel carbonyl, added as anti-knock to motor fuels, can be detected by an analogous procedure. Ammonia water is used to take up the metal and a 1 per cent alcohol solution of dimethylglyoxime is the reagent.

Chapter VIII

SPOT REACTIONS IN BIOLOGICAL MATERIAL

The biological sciences (physiology, medicine, pharmacology, etc.), present numerous tasks and problems whose prosecution and successful solution require chemical methods of investigation. Extensive and clear cut information concerning the presence or absence of certain materials, their localization, action and alteration, must usually be secured, since important biological conclusions can often be drawn from such data. Inorganic as well as organic compounds may be involved. Frequently in studies of biological materials, the subject of special interest is not the framework (stromatic material) of the vegetable or animal cells. The material of prime importance is rather the determination of the kind and quantity of the materials contained in the cell. These materials are present in far smaller quantities.

From the analytical standpoint, the problems of biological nature that are to be treated chemically are, for the most part, essentially microchemical in their nature, or microchemical methods are particularly advantageous because of economy of time and material. Accordingly, many advances in the field of biochemistry have been closely connected with the development of microchemical methods. Consequently, when chemical studies are made with biological materials, it is necessary to keep in mind the limits of sensitivity and definiteness of the present macro- and microchemical methods. In most cases it is useless to attempt to study, by chemical methods, problems dealing with changes in form and material in the individual cells, because the absolute quantity of the materials involved is too minute. On the other hand, when studying and following chemical processes in groups of cells, that is, in tissues, parts of organs, and in extracts and body fluids, it is frequently possible to detect and determine essential constituents, even though these occur in very small amounts.

The biological sciences present a series of problems involving the search for traces of organic and inorganic compounds. As in inorganic analysis, the use of catalyzed and induced reactions affords the greatest possible sensitivity in the detection of organic compounds. The material to be detected serves as the catalyst or inductor. When such reactions are used for chemical tests, the analytical study employs the same reaction mechanism by which many compounds of the living organism are produced or transformed. The use of spot reactions in the study of biological problems is of recent date, but the useful findings that have been secured thus far show definitely that spot analysis can be applied successfully in this field also.

1. Examination of Drinking Water for the Presence of Lead

Lead may occur in streams and in underground waters in mining regions. It also is sometimes present in soft water areas, because such water may become contaminated from the lead piping, since this metal readily dissolves in soft water through the action of the air and carbon dioxide.

Any examination of water for its potability should include a test for lead, because small quantities accumulate in the human organism and may result in a toxic condition. All water containing more than 0.3 mg. Pb per liter is unfit for human consumption. The standard methods of water analysis prescribe a quantitative determination of lead. When carried out properly, this requires 3 to 4 liters of water, a number of auxiliary reagents, and consumes about two days.

The procedure given here does not pretend to be quantitative. It will, however, reveal, by a color reaction, any lead content exceeding 0.5 mg. per liter. Not more than 10 ml. of water is necessary, and the test can be completed in 30 to 40 minutes. The method suffices to disclose waters that should be rejected because they carry excessive amounts of lead. To reveal quantities between 0.25 mg. and 0.5 mg. Pb per liter, a given volume of water can be reduced to half its volume by evaporation, and the test then carried out on 10 ml. of the concentrate. A positive reaction then indicates the presence of 0.25 mg. Pb per liter; a negative result shows that the lead content is below this level.

Chemical Basis: Traces of lead that are too small to be precipitated by hydrogen sulfide can be coprecipitated by mercuric sulfide. The HgS acts as a "trace catcher" or accumulator. If this mixed precipitate is dried and ignited, the mercuric sulfide is completely destroyed and volatilized. Only the lead remains, and this, now transformed to sulfate, forms a very fine suspension. Under these conditions, the lead sulfate reacts immediately with sodium rhodizonate solution and forms a colored salt (see p. 135).

The procedure given here will *permit the detection* of 5 γ Pb in 10 ml. of water; if concentrated to half its volume, 2.5 γ Pb can be detected. These results correspond to 0.5 mg. and 0.25 mg. Pb per liter, respectively.

Procedure: Ten milliliters of the water are placed in a test tube and acidified with one drop of concentrated hydrochloric acid. Twenty milligrams of mercuric chloride are introduced and the solution is then treated with hydrogen sulfide until the precipitation is completed. The precipitate is collected on a paper, dried, and then transferred to a small crucible and heated cautiously. When the mercuric sulfide has been driven off, the crucible is ignited for several minutes. After cooling, the residue is treated, in the crucible, with three drops of buffer solution (see p. 217) and one drop of freshly prepared (0.2 per cent) sodium rhodizonate solution.

If the lead content is less than 5 γ, a discoloration appears in 2 to 3 minutes; when 5 γ or more is present, red flecks appear.

If no reaction takes place, the test may be repeated with the same water after it has been concentrated to one-half its volume. A positive reaction in 10 ml. of the concentrated water indicates the presence of over 2.5 γ Pb, corresponding to 0.25 mg. Pb per liter in the original water.

Water containing flecks of hydrous ferric oxide or other suspended materials must not be filtered, because all such materials retain lead by adsorption. Even filter paper holds back lead from solutions passed through it.

2. Detection of Potassium in Blood, Saliva, Animal and Vegetable Tissues

Chemical Basis: Formation of potassium dipicrylamine (see p. 115).

Procedure: A drop of saliva, blood, etc., is placed on filter paper impregnated with sodium dipicrylamine. After drying in a blast of hot air, the spotted paper is bathed in dilute nitric acid until the paper becomes bright yellow (color of the free dipicrylamine). The paper is then placed in water to avoid needless prolonged action of the acid. When potassium is present a red fleck or ring will be left.

The *identification limit* is 3 γ potassium.

If only minute quantities of potassium are expected, it is advisable to evaporate several drops carefully on platinum foil. The residue is ignited, cooled and taken up in a macrodrop of water. Microdrops of the solution are transferred to the reagent paper by means of a glass capillary.

When animal or vegetable tissues are to be tested for potassium, an appropriate quantity is ashed. The ash is treated with water and a drop of the suspension (no need to filter) is placed on the reagent paper.

3. Detection of Traces of Copper in Biological Media

If water is put in contact with metallic copper (or silver) a definite germicidal action will be evident within a short time. This "oligodynamic effect" is due to traces of copper (silver) which enter the water as ions. They probably act on the albuminous material of bacteria to form copper (silver) compounds.

The presence of copper ions sometimes brings about important biological effects, because the reversible change $Cu(II) \rightleftharpoons Cu(I)$ can catalytically accelerate either oxidation or reduction reactions. A model inorganic instance of this action of copper is the oxidation of sulfite solution when exposed to the air. This auto-oxidation is much slower in water containing no copper than it is in ordinary water containing traces of this element.

Consequently, in biological studies there is often a real reason for testing water or other material for traces of copper. This test can be made by

the procedure given here. The effect can also be used to prove that traces of copper go into solution when water is brought in contact with metallic copper or with alloys containing a little copper.

Chemical Basis: Catalytic acceleration of the $Fe^{+++} - S_2O_3^{--}$ *reaction by copper salts.* The reaction

$$2\ Fe^{+++} + 2\ S_2O_3^{--} = 2\ Fe^{++} + S_4O_6^{--}$$

is catalyzed by copper ions. Accordingly, if a solution of red ferric thiocyanate, which always contains ferric ions:

$$Fe(CNS)_3 \rightleftharpoons Fe^{+++} + 3\ CNS^-,$$

is treated with a solution of sodium thiosulfate the color fades after some time. This fading period is shortened if even traces of copper salts are added (see p. 161). The preparation of the reagent solutions is described on p. 161.

The *identification limit* of this test is 0.02 γ copper in drops.

Copper can be detected in much more dilute solutions if the solutions are shaken with an adsorbent for copper. The adsorbent is then isolated and brought in contact with a $Fe^{+++} - S_2O_3^{--}$ solution. A distinct difference in the decolorizing periods will be observed on comparison with a parallel test with the pure adsorbent.

If vegetable or animal tissues or pharmaceutical products are to be tested for traces of copper, a sample should be ashed and the residue spotted with the reagent solution.

(a) Demonstration of the Solubility of Metallic Copper

One drop of $Fe(CNS)_3$ solution and one of $Na_2S_2O_3$ solution are brought together in adjacent depressions of a spot plate. One mixture, for comparison, is left untouched or is stirred with a wooden splint (toothpick). The other is stirred with a copper wire or a gold ring. The color disappears almost immediately after the copper wire comes in contact with the solutions. The fading also is distinctly quicker after contact with the ring, since gold is always alloyed with small quantities of copper.

(b) Detection of Copper at a Dilution 1:1,000,000,000

A copper sulfate solution containing 1 γ copper per liter is prepared by step-wise dilution. Ten milliliters of this $1:10^9$ solution are placed in a hard glass test tube provided with a stopper. About 10 mg. of calcium fluoride or talc is added and the suspension is shaken for 30 minutes on the shaking machine. For comparison, an equal volume of doubly distilled water is similarly treated with the same quantity of adsorbent. The suspensions are then centrifuged. Approximately equal quantities of the

two adsorbents are transferred to adjacent depressions of a spot plate. Single drops of $Fe(CNS)_3$ and $Na_2S_2O_3$ solutions are added in turn. (The definiteness of the observation is increased if the thiosulfate is added to the blank test before it is added to the copper solution.) It will be found that decolorization occurs at different rates; it is faster with the adsorbent that was shaken with the water that contained copper.

This procedure can be used to detect traces of copper in samples of water. It is advisable to run a comparison test with water known to be free of copper. This can be prepared best by shaking a liter of doubly distilled water for 2 hours with 2 g. of talc. The adsorbent is allowed to settle and the necessary quantity of water is taken each time from the storage vessel.

(c) Detection of Copper in Plant and Animal Tissues

Varying quantities, determined by trial, of the material to be tested are ashed in a quartz crucible. The cooled residues are treated with 2 drops each of ferric thiocyanate and thiosulfate solutions. Traces of copper can be detected by the shortening of the decolorization period in comparison with the time required in parallel tests.

4. Detection of Reducing Sugars

Chemical Basis: The reduction of silver oxide to metallic silver. Alkaline solutions of reducing sugars act on finely divided freshly precipitated silver oxide even at room temperatures and produce free silver. Since ammonia dissolves silver oxide and, in contrast, not more than traces of metallic silver, the reduction can be carried out as a spot reaction on paper in such a manner that the metallic silver is fixed on the paper. Minute quantities can thus be made plainly visible.

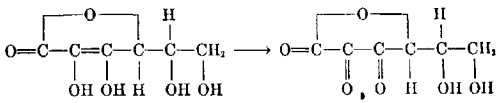
The detection of reducing sugars with the aid of silver oxide is distinctive only when other reducing compounds are absent. For instance, silver oxide is reduced, under the conditions outlined here, by tartaric acid. The reaction cannot be applied to detect sugar in urine, because of the presence of uric acid, also a reducing agent. Compounds with —SH or —CS groups should be absent, since such materials may produce black silver sulfide, which is not soluble in ammonia.

Procedure: Strips of filter paper are bathed in 0.2 *N* silver nitrate solution immediately before making the test, and dried in a blast of heated air. One drop each of the alkaline test solution and of a sodium hydroxide solution of about the same strength are placed next to each other on the silver nitrate paper. Brown flecks of silver oxide appear immediately. After about one minute, the paper is placed in 6 *N* ammonia. The pure silver oxide fleck disappears almost completely in a short time, whereas the fleck formed by the specimen containing reducing sugar forms a black to bright brown spot or circle according to the quantity of sugar present.

Identification Limit: 0.1 γ sugar.
Concentration Limit: 1:500,000.

Detection of Ascorbic Acid (Vitamin C)

Chemical Basis: Reduction of manganese dioxide or ammonium phosphomolybdate. Ascorbic acid (I) is a strong reducing agent that is oxidized to dehydroascorbic acid (II) by MnO_2 or $(NH_4)_3PO_4 \cdot 12\,MoO_3$:



(I) (II)

These two oxidizing agents are not soluble in water. They can be precipitated in the capillaries of filter paper, where, because of their fine state of subdivision, they are so reactive that very small quantities of ascorbic acid can be detected by spot reactions by the disappearance of MnO_2 or by the formation of molybdenum blue (see p. 102).

Manganese dioxide paper is prepared by soaking filter paper for 15 minutes in a solution obtained by diluting 1 ml. of 0.2 *N* $KMnO_4$ to 1000 ml. After draining, the paper is dried in a blast of heated air. Some of the cellulose of the paper is oxidized; the MnO_2 formed remains in the capillaries. The paper is brown to practically colorless, according to the quantity of manganese dioxide present. Larger quantities of ascorbic acid can be detected directly by the formation of a white fleck when a drop of the test solution is placed on the brown paper. Almost colorless reagent paper should be used for revealing the presence of very small quantities of ascorbic acid. The consumption of manganese dioxide in this case is made visible by placing the paper in a benzidine solution prepared by diluting a saturated solution of benzidine hydrochloride with an equal volume of water before using. Traces of manganese dioxide form benzidine blue (see p. 141). Consequently the whole paper will become blue, except those portions reduced by the ascorbic acid.

Phosphomolybdate paper is prepared by bathing filter paper in a saturated alcohol solution of phosphomolybdic acid. The paper is drained, dried in a blast of cold air, and then bathed in a concentrated solution of ammonium nitrate that has been acidified with several drops of nitric acid. After washing with water, the paper is dried in a blast of air. It will keep for several days if stored in the dark. The paper must be rather fresh, since it gradually turns green, particularly in the daylight, because of reduction by the cellulose.

Procedure (on MnO_2 paper): A drop of the test solution, which may be neutral, alkaline, or weakly acid with acetic acid, is placed on the reagent

paper. After the drop has soaked in, the paper is bathed in benzidine solution. When ascorbic acid is present, a white fleck is left on the blue paper.

It should be noted that many reducing compounds, such as citric acid in weakly acid solution, react in this same way on manganese dioxide. To detect ascorbic acid when citric acid is also present, in fruit juices for instance, the test solution is first shaken with an excess of calcium carbonate. A drop of the suspension is then placed on the reagent paper. The reduction of manganese dioxide by citric acid is completely prevented while the action of ascorbic acid is unaffected.

Identification Limit: 0.03 γ ascorbic acid (in 0.004 ml.).

Concentration Limit: 1:130,000.

Procedure (on $(NH_4)_3PO_4 \cdot 12\ MoO_3$ paper): A drop of the acid, neutral, or alkaline test solution is placed on the reagent paper. A blue or green fleck appears on the yellow paper immediately or after several minutes, depending on the quantity of ascorbic acid present.

Citric acid does not react under these conditions, so that 1 part of the ascorbic acid can still be detected even though 1000 parts of citric acid are present.

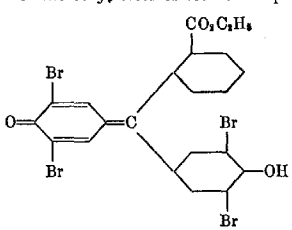
When testing urine for ascorbic acid, the specimen must be treated beforehand with several drops of concentrated hydrochloric acid to prevent the uric acid from reducing the ammonium phosphomolybdate. The reaction of uric acid and urates with ammonium phosphomolybdate is very sensitive and can be used for the detection of these compounds.

Identification Limit: 0.1 γ ascorbic acid (in 0.01 ml.).

Concentration Limit: 1:100,000.

5. Detection of Native Albumin

Chemical Basis: Reaction with the ethyl ester of tetrabromophenolphthalein. The yellow solution of the ethyl ester of tetrabromophenolphthalein



acts as an indicator. In alkaline solution it is deep blue; on addition of dilute acids (even acetic) the yellow color is restored. Albumins also

produce a blue, which, however, is stable toward dilute acetic acid; it disappears only on the addition of concentrated acetic acid or of mineral acids. The indicator combines with albumin and forms a salt-like compound, which is stable toward dilute acetic acid. If, therefore, a blue solution of the alkali salt of tetrabromophenolphthalein ethyl ester is treated with a solution of albumin, the blue persists on acidification with dilute acetic acid.

The reaction is specific for native albumin. Scission products of albumin, such as amino acids, di- and tripeptides, or peptones do not react. Alkaloids of high molecular weight, in considerable concentration, simulate this reaction.

Procedure: The reagent is an 0.1 per cent alcohol solution of the potassium salt of tetrabromophenolphthalein ethyl ester. A drop of the solution to be tested for albumin is treated on a spot plate, or in a microcrucible, with a drop of the reagent. The mixture is acidified with 1 or 2 drops of 0.5 *N* acetic acid. The color of the blank test becomes light yellow, while in the presence of albumin, a more or less intense blue appears. If only small quantities are involved, the solution remains greenish.

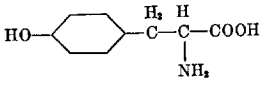
The following quantities can be detected by the foregoing procedure:

0.5 γ egg albumin	5 γ edestin
0.5 γ hemoglobin	0.5 γ clupein
0.35 γ serum albumin	0.5 γ salmine
0.5 γ casein	1 γ gliadin

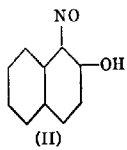
A pathologically increased quantity of albumin can be detected in one drop of urine by means of this reaction.

6. Detection of Tyrosine

Chemical Basis: Reaction with α-nitroso-β-naphthol. Tyrosine (I) and albuminous materials on treatment with α-nitroso-β-naphthol (II) and nitric acid produce a dark purple color:



(I)



(II)

The mechanism of the reaction has not been clarified; the nitric acid seems merely to oxidize the α-nitroso-β-naphthol to α-nitro-β-naphthol, because it may be replaced by either manganese dioxide or lead dioxide.

The reaction is specific for tyrosine in the presence of other amino acids, 3,4-dihydroxyphenylalanine, adrenaline, thyroxine, aldehydes, sugars, and urea. Similar reactions are given by many para-substituted phenols such

as tyrosol, thyramine, *p*-cresol, *p*-ethylphenol, hydroquinone monomethyl ether, β-naphthol, phenolphthalein, 1,2,4-xylenol, *p*-chlorophenol, *p*-bromo-*m*-cresol.

To detect tyrosine in pathological urine or serum, 0.5 ml. of the specimen is mixed with 1 ml. of water and then treated with 1 ml. of 20 per cent trichloroacetic acid. The mixture is well stirred and the precipitated albumin is separated by centrifuging. The test is made on one drop of the centrifugate.

Procedure: A drop of the solution to be tested for tyrosine is treated in a microcrucible with 1 drop of 0.2 per cent alcohol solution of α-nitroso-β-naphthol. The mixture is heated and a drop of concentrated nitric acid is added to the hot solution. When tyrosine is present, a purple color appears. It fades after a few minutes.

Albuminous materials almost invariably contain tyrosine and therefore can be detected easily by this reaction. They are conveniently hydrolyzed beforehand by heating with sodium hydroxide or sulfuric acid.

Identification Limit: 0.05 γ tyrosine.

Concentration Limit: 1:1,000,000.

7. Detection of Enzymes

A number of inorganic substances may often be detected in extremely small amounts by their catalytic action, either by recognizing a product of the catalysis or by the disappearance of one of the participants in the reaction. Such tests for the catalytic substance are nearly always specific. The following test for certain enzyme groups present in the organic compounds of plant or animal material, rests on the same principle, when the enzyme plays an important part in certain chemical processes in the biological material. Such enzymes (bio-catalysts) are distinguished by the changes they cause in definite substrates, such changes as oxidation, reduction, change of hydrogen ion concentration, formation of characteristic compounds, etc. By the use of sensitive tests and very small amounts of substrate these changes may be recognized and hence the enzymes themselves identified.

Procedure: A drop of the substrate solution and a drop of the solution to be tested for a particular enzyme are mixed on a strip of filter paper or in a microcrucible. Both the sample and a blank test (a drop of water and a drop of the substrate) are heated for 40–60 minutes, under a cover glass to keep in the moisture, and the product of the reaction treated with a suitable reagent.

In the accompanying table a summary of the tests for a few enzymes are given.

Other reactions, described elsewhere in this book, may also be applied as tests for enzymes. Some examples will be given.

(a) Urease

Urease causes urea to break up into ammonia and carbon dioxide. Small quantities of ammonia can be detected by the precipitation of the black adsorption compound $MnO_2 \cdot 2Ag$ from a manganous-silver solution. Urease can therefore be detected by mixing a drop of the enzyme solution and a drop of 10 per cent solution of urea in the apparatus described on p. 51 (Fig. 28). The funnel stopper is covered with a piece of filter paper impregnated with manganous-silver solution (preparation see p. 202). When urease is present, the paper turns black in about 10 minutes due to the action of the ammonia liberated.

Suggested test material: soya beans.

TABLE II

Enzymes

Enzyme	*Substrate*	*Reagent*	*Color Change*
Diastase	0.5% soluble starch	Fehling's solution	brick red or orange
Inulase	0.5% inulin	" "	" " " "
Invertase	0.5% cane sugar	" "	" " " "
Emulsin	0.5% salicin	" "	" " " "
Emulsin	0.5% indican	treatment with alkali with air blown through	blue
Lipase	0.2% emulsion of olive oil	methyl red	red
Butyrase	0.2% ethyl acetate-water emulsion	" "	red
Urease	1% urea	phenolphthalein	rose
Phenolase	1% tincture of guaiac	"	blue
Tyrosinase	tyrosine	"	brown

(b) Zymase

The zymase system ferments sugar with the formation of alcohol and carbon dioxide. The latter may be identified by the reaction described on p. 107 (decolorization of sodium carbonate solution colored red with phenolphthalein). The test can be carried out in the apparatus described on p. 50 (Fig. 26). A drop of the solution to be tested for zymase is mixed with a drop of 5 per cent solution of glucose. The carbonate solution is decolorized in about 10 minutes when zymase is present.

Suggested test material: yeast.

(c) β-Glucosidases (Emulsin)

Emulsin causes amygdalin (a glucoside) to break down with liberation of prussic acid. The latter can be easily identified by the cyanide test described on p. 145 (blue color with benzidine-copper acetate).

A drop of the solution to be tested for emulsin is placed in the apparatus described on p. 51 (Fig. 28). A little amygdalin is added and the funnel stopper is covered with a piece of filter paper impregnated with benzidine-copper acetate reagent (see p. 146). A blue color, after a few minutes, indicates prussic acid, and hence emulsin.

Suggested test material: bitter almonds.

(d) Lipase

Lipases are ferments that split esters. They can be detected by the test for esters described on p. 187 (formation of ferric hydroxamate).

Single drops of castor oil emulsion (about 0.3 per cent) are placed in two microcrucibles. A drop of the solution to be tested for lipase is put into one of the crucibles. The crucibles are kept at 35°C. in the drying oven for 1 to 2 hours. The contents are then tested for esters. When lipase is present the reaction is negative or decreased in intensity, while the blank test gives the violet characteristic of esters.

Suggested test material: mucous membrane of a hog's stomach.

(e) Proteases

Proteases are albumin-splitting ferments which break down native albumin to lower polypeptides. They can be detected easily by using the albumin reaction described on p. 238 (formation of blue compounds with the ethyl ester of tetrabromophenolphthalein).

Single drops of an aqueous suspension of a little casein or egg albumen are placed in two microcrucibles. A drop of the solution to be tested for protease is added to one of the crucibles, a drop of water to the other. The crucibles are kept at 35°C. for 1 to 2 hours in the drying oven. The solutions are then subjected to the albumin reaction. When protease is present, the response is negative or far less intense, while the blue color characteristic of albumins is given by the blank test.

Suggested test materials: pepsin or trypsin.

(f) Catalases

Catalases are ferments which speed up the decomposition of hydrogen peroxide. They can thus be detected by the disappearance of the peroxide from the substrate. The reaction (p. 109) with lead sulfide (white fleck on PbS paper) can be used to indicate the presence or absence of hydrogen peroxide.

A drop of the solution to be tested for catalase is treated in a microcrucible with a drop of 3 per cent hydrogen peroxide solution. A parallel test is made with one drop of the hydrogen peroxide solution. After an hour, a drop from each of the crucibles is placed on lead sulfide paper.

When catalase is present, the hydrogen peroxide reaction fails or is much weaker than in the blank test.

Suggested test material: fresh meat.

(g) Peroxidase

The hemoglobin of blood has the activity of a peroxidase; it catalyzes the oxidation by hydrogen peroxide of benzidine to benzidine blue.

A drop of the solution (urine, for instance) to be tested for blood is placed on spot paper (S. and S., 598 G) and treated first with a drop of 3 per cent hydrogen peroxide and then with a drop of 0.05 per cent solution of benzidine in 10 per cent acetic acid. When blood is present, a blue stain appears after a time, that varies from a few seconds to one minute, according to the amount of blood present. The color lasts for about one hour.

The sensitivity and rapidity of the test may be increased, but at the expense of the length of the life of the color, by adding a drop of 2 *N* sodium hydroxide to the spot paper before carrying out the test.

Chapter IX

SPOT COLORIMETRY

The use in analysis of colored precipitates or colored soluble reaction products formed by spot reactions is not limited to the detection of various inorganic or organic compounds, ions or radicals. Such spot reactions may also serve as the basis of quantitative methods based on the following principle of color comparison:

The compound whose quantity is to be determined, is used to prepare a series of standard solutions, that is, solutions of the pure material in known concentrations. Suitable spot reactions are carried out with a drop of each of these standard solutions, on a spot plate or on filter paper. A series of colored drops or flecks of different intensities is thus obtained. The same spot reaction is then made on the same medium with the drop of the test solution, whose content is being determined. The intensity of the color produced is compared against the color scale prepared from the standard solutions. It is not difficult to tell with which spot of the standard series the sample agrees. Therefore the quantity of material involved can be determined.

This method of quantitative determination by comparison of the color intensities produced by spot reactions is known as *spot colorimetry*. It has been used almost exclusively for the quantitative determination of inorganic materials. The subject can be considered in two categories, depending upon the procedure employed. In the first, the reaction is carried out with a drop of the test solution on the spot plate. In the second, the reaction occurs on filter paper. Accordingly, it is common practice to distinguish between:

Spot colorimetry by comparison of colored solutions on non-porous substrates.

Spot colorimetry by comparison of spots (flecks) on filter paper.

In addition to the method of direct color comparison there is another procedure in which spot reactions are used as directive quantitative determinations. It is based on the fact that every method of making a chemical test, and this includes spot reactions, gives clearly visible results only if the quantity of material to be detected is not below a certain lower limit: the identification limit (see p. 2). Consequently, if the identification limit of a color reaction performed as a spot test is known, a given test solution can be progressively diluted with constant testing of drops taken at the various dilutions, until the color reaction fails. The concentration of the original solution can then be calculated from the dilution required to reach this limiting concentration. For instance, if a certain spot reac-

tion has an identification limit of 0.3 γ, in drops, and the test solution must be diluted to $\frac{1}{120}$ before the reaction is no longer visible, then one drop of the original test solution contained $120 \times 0.3\ \gamma = 36\ \gamma$ of the active material. The repeated dilution and testing of drops required by this procedure is time-consuming and the results are only approximate. It is inexact because the examination for the failure of a test always extends into the region of uncertain reaction (see p. 4). Therefore it is vitiated with a fundamental fault which cannot be eliminated. Furthermore, even slight experimental errors become quite significant, because the subsequent computation involves a multiplication. Consequently, quantitative determinations based on the ascertainment of the detection limit are of far less practical importance than the spot colorimetric determinations made by comparing the depth of color of solutions and flecks.

A. Observations on the Making of Spot Colorimetric Determinations

The volume of the test solution as well as the size of the drops of the test portion and of the standard solutions being compared must be known in all spot colorimetric determinations. These data are essential because the actual performance of the spot reactions and the comparison of the colors must always be followed by a calculation back to the volume of the original test solution or to the quantity of material dissolved in it.

The size of the drops can be measured either by means of the iodine pictures (see p. 64) or the solutions can be placed in separate microburettes (1 ml. capacity) and the drops required for the spot reactions taken from these. The volume of the drops delivered by these burettes can, of course, be read directly.

The microchemical and semi-microchemical nature of spot colorimetric determinations, as a rule, permits the use of less than three milliliters of a test solution for the execution of about ten comparison determinations. Consequently, dilute solutions may be evaporated to a small volume, and then placed in small calibrated flasks (capacity about 5 ml.). If necessary, too concentrated solutions can be suitably diluted.

The most important detail in all spot colorimetric determinations is that the spot reactions performed with drops of the standard and of the test portions must be carried out under exactly the same conditions, as far as possible. In practice, precisely identical conditions can be maintained only in the rather uncommon case when pure solutions are used. In general, the presence of varying quantities of accompanying materials must be taken into account. If the nature of these materials and their approximate quantity is known, the same materials in about the same proportions should be added to the standard comparison solutions. This

step is absolutely necessary in determinations from which particularly accurate results are desired, because the presence of apparently indifferent materials can often affect the reaction picture of a chemical change. This is because the reaction of small quantities of a material may be retarded by large amounts of accompanying materials; in fact, the reaction is entirely prevented at times (see p. 13).

The choice of the color reaction to be used in spot colorimetry is always determined by the individual problem. In general, quantitative determinations, provided they are not control determinations of thoroughly familiar products, are never carried out until the qualitative composition of the specimen has been established. The operator will then have a knowledge at least of the nature of the accompanying materials and can choose such spot reactions as are specific and subject to no, or only slight, influence by these other materials. Sometimes interfering materials can be masked by isolating them as complexes (see p. 148). In such cases, it is also necessary to mask the standard solution before carrying out the spot reaction.

The choice of the color reaction is also made after ascertaining whether considerable quantities or only traces of the material to be determined are present in the solution. Since several test reactions of different sensitivity are available for many compounds and are also suitable for colorimetric determinations, it is easy to choose one that is appropriate for either larger or smaller quantities of the material being determined. As a rule, the less sensitive color reactions will be used when dealing with relatively large amounts, and highly sensitive color reactions will be reserved exclusively for very small quantities.

In all color reactions there are concentration limits of the colored reaction product above which it is difficult to discern equal intensities or slight differences in intensities when the color of small volumes of liquid are compared on a spot plate or in flecks on paper. In general, differences in color intensity are best appreciated at medium and lower concentrations. The particular color itself is a factor. For this reason it is well to carry out a preliminary orienting color reaction with the test solution, so that after comparison with the color produced by the standard solution, an approximate idea will be had as to which concentration range is best suited for an accurate comparison. The test solution can then be brought into conformity with these findings, either by proper dilution or evaporation. The accurate comparisons are then made with drops of the diluted or concentrated solution.

A series of decreasing concentrations is prepared from the standard solutions; each member contains one half the quantity of dissolved material present in the preceding solution. After it has been found that the concen-

tration of the test solution lies between those of two successive dilutions, two or three dilutions within this range are prepared from the standard solution. The accuracy of spot colorimetric determinations is raised considerably by this procedure.

It is not necessary to use calibrated vessels when diluting the test and comparison solutions for mere orientation trials. The following method can be used: The first depression of a spot plate is filled with the solution to be diluted. Three drops are transferred to the next depression and mixed there with 3 drops of water. The mixing is accomplished by blowing through a pipette. Three drops of this 1:1 solution are placed in the next depression, diluted with 3 drops of water and mixed. This progressive dilution is repeated several times more. Drops of each of these diluted solutions can be taken for the spot reaction to determine the dilution most suitable for the actual colorimetric determination. The corresponding dilution for the quantitative measurement can then be made accurately in calibrated flasks.

Spot colorimetric determinations give the best results when a color reaction is accomplished by bringing together one drop of the test portion and one of the reagent, or when a drop of the test portion is allowed to react directly on the solid reagent. This simple procedure is not possible with all color reactions, since neutralization, alkalization, acidification, etc., are often required. These preparative or additional steps must always be carried out in the same way, that is, in the same order and with the same quantities of auxiliary and main reagents both on the test solution and on the standard comparison solution. Special attention must be paid to the fact that sometimes the full intensity of color reactions is attained only after a certain period of time has elapsed. It is also important to know that many colored products are not stable. Consequently, the spot reactions of the standard and the test solution should be made directly after each other, and the result of the reactions compared not merely once, but also after several time intervals. This also is easier if a preliminary orientation test has indicated the approximate concentration range most suitable for the comparison.

Color reactions in colorless solutions with colorless reagents should be used whenever possible. If this condition cannot be met directly, that is, if the test solutions or the reagent are colored, nonetheless, this handicap, within certain limits, does not present an insuperable barrier for satisfactory spot colorimetric determinations. These measurements never involve the determination of an absolute color, but require merely a comparison of colors, which may include mixed colors. Since the determination of a change in color is more difficult to evaluate in colored solutions than in colorless or only faintly colored surroundings, an effort should be made to

use dilutions of both the test and comparison solutions at which the matching of the colors is less influenced by the accompanying colored materials.

Quantities of material ranging from 0.1 to 300 γ can be determined in one drop of the test solution by spot colorimetry. This signifies that quantitative determinations can still be made in solutions of 0.0007 to 0.6 per cent. The order of magnitude of the quantity that can be determined, accordingly, places these measurements in the class of microchemical or semi-microchemical determinations.

As to the reliability of spot colorimetric determinations, the accuracy of the results depends on the kind and quantity of the material being determined, the correct choice of the color reaction, the care with which the procedure is followed, and also the experience of the operator. It is obvious that the accuracy of these determinations, in general, is below that obtained from colorimetric determinations made with the aid of optical instruments. A deviation of $\pm$ 10 to 20 per cent from the theoretical value can be expected in spot colorimetric determinations involving a comparison of turbidities or colors produced in drops of the test solution. Far better results are furnished by comparing color flecks on paper; if special precautions are taken, the deviation from the actual value can thus be reduced to 5 to 10 per cent.

It is always important to be cognizant of the possibilities and limits of application of analytical methods. This holds also for spot colorimetry: its goal can never be precision analyses, but it furnishes an excellent estimate of the quantity of material present in a solution in a shorter time than is possible by other methods. This type of orientation is fully adequate for many purposes, such as testing of materials, process control, etc. It frequently can be used in place of an exact analysis or it may furnish important directive information if such an analysis is to be made later. The advantages of spot colorimetric determinations are: the simplicity of the procedure, the small quantity of material required, economy of time. Furthermore microchemical and semi-microchemical determinations can be made far more quickly by this procedure than when performed by the classical methods of microanalysis. In many cases the disadvantage of a lesser accuracy is fully compensated by the rapidity with which the determination can be made.

B. Spot Colorimetry by Comparison of Colored Solutions

The first spot colorimetric determinations based on the comparison of the intensity of color reactions produced by drops of a test solution on a spot plate or on filter paper were described about 1928 by the Russian chemist N. A. Tananaeff. Since then this procedure has been repeatedly recommended, particularly in Russian publications, for the rapid estimation of

important constituents of minerals and for the chemical control of technical processes.

Spot colorimetry using comparison of small quantities of colored materials on a spot plate, or some other non-porous medium, are based on the premise that spot reactions give either colored soluble reaction products or that, after a given interval, a colored slightly soluble material is still homogeneously suspended throughout the solution. This latter condition is often encountered when very small quantities of such compounds are formed, since these undergo a gradual aggregation and hence must pass through the range of colloidal dispersion and thus remain, temporarily at least, in apparent solution. Occasionally even relatively large quantities of slightly soluble compounds can be kept colloidally dispersed for considerable periods by adding protective colloids, such as gelatin, ethyl cellulose, etc. Sulfides and other insoluble compounds can be peptized by such additions, and the resulting colloidal "solutions" used for spot colorimetric determinations. It is well, in such cases, before starting the chemical reaction, to add the protective colloid either to the entire test portion or to the drop of this taken for the test. The same peptization must, of course, be applied to the standard solution. No general directions for peptizing precipitates can be given. Special trials must be made to discover whether a particular precipitate can be peptized, since success often depends on the kind and quantity of the other materials present.

Spot colorimetric determinations based on the comparison of the color of solutions can be made with colored true solutions or with colored colloidal solutions on white porcelain spot plates or on spot plates made of glass. If the latter are used, the matching of the colors is often facilitated by placing white or colored paper under the transparent plate.

In general, such color reactions as go to completion even at room temperature should be used in spot colorimetry. Any heating, to which of course the comparison solution must also be subjected, invariably introduces a factor of uncertainty with respect to the equality of the treatment. If heating cannot be avoided, the spot reactions should be made in microcrucibles; during the heating these are set next to each other on either an electric hot plate, a heated asbestos mat, or a water bath.

Stable colored suspensions (turbidities) can be compared with reference to the quantity of suspended material in the same way as colored solutions are matched. Actually these are not colorimetric determinations; they are really nephelometric determinations, which, however, are entirely analogous to color determinations in this technic. The formation of white or bright yellow precipitates which can form fairly stable suspensions ($BaSO_4$, $BaCrO_4$, ZnS, etc.) may be used as the basis for spot colorimetric, or more correctly spot nephelometric determinations. Black spot plates

should be used in such cases, or glass spot plates set on glazed black paper; in both instances it is easy to compare the intensity of the turbidities. Spot nephelometric determinations, in contrast to spot colorimetric measurements, must always be made very soon after bringing the reactants together, to forestall the aggregation and sedimentation that invariably slowly ensue.

As a rule, the characteristic chemical reactions used in spot colorimetry are carried out with drops of the test and comparison solution. However, color reactions can be made with a larger volume of the solutions, several milliliters for instance, and then drops of the resulting colored solutions are taken out and matched on a spot plate. This procedure requires the formation of very stable colored solutions. It permits also the preparation of colored standard solutions, of which one or two drops can be taken for the comparison.

Spot colorimetric determinations made on a spot plate require that the union of the drops occur at the bottom of the depressions; losses should not be incurred, for instance, through wetting of the walls or hanging of part of the solutions. Consequently, the depressions of the plate must be kept free of grease by wiping them with cotton soaked in alcohol or ether. It is never permissible to mix the solutions by stirring them with a glass rod; this inevitably leads to loss. The proper method is to blow on the drops from one side through a pipette. Since very small quantities of liquid are involved in these determinations, every slight loss produces rather significant errors in the results, and consequently, the greatest care is imperative in this type of work.

The essential details of the technic are given in the foregoing and under the heading "Observations on the Making of Spot Colorimetric Determinations" (see p. 245). It is extremely important to acquire practical experience by repeated trials. The following are recommended as practice exercises.

Determination of:

1. Iron (III) salts by formation of $Fe(CNS)_3$....red solution.
2. Iron (II) salts by formation of $Fe(\alpha,\alpha'\text{-dipyridyl})_3^{++}$ ions....red solution.
3. Iron (III) and iron (II) salts in the presence of each other by use of 1 and 2.
4. Copper by formation of $Cu(NH_3)_4^{++}$ ions....blue solution.
5. Chromate by formation of chromate-diphenylcarbazide....violet solution.
6. Barium or sulfate by formation of barium sulfate....white suspension.

7. Barium by formation of barium chromate....yellow suspension.
8. Titanium by formation of Ti(IV)-chromotropic acid....violet solution.
9. Silicic acid (silica) by formation of silicomolybdic acid....yellow solution.
10. Traces of copper by decolorization of $[Fe(CNS)_3 + Na_2S_2O_3]$ solution (see p. 161).

This is an example of a chronometric determination.

C. Spot Colorimetry by Comparison of Colored Spots on Filter Paper

There are two methods of conducting spot colorimetric determinations based on the comparison of colored flecks on filter paper. The older procedure is to bring drops of the test and comparison solutions into contact with drops of the reagent on filter paper and to compare the intensity of the colored spots. The second procedure has been developed since 1937 by the American chemist H. Yagoda; it represents a considerable advance because it can furnish much more accurate results. The spot reactions are carried out on reagent papers with limited reaction surfaces and are followed by a colorimetric comparison.

If drops of solutions of reactants are united on filter paper, the reaction (spot) picture is determined by a number of factors. In addition to the purely chemical changes, adsorption phenomena, capillary and diffusion processes, characteristics of the paper, and other considerations play so important a part that the "spot picture" is always the composite of a combination of concurrent and subsequent chemical and physical changes (see Chapter III). Consequently, a comparison of the intensities of colored flecks on paper is only well suited for the estimation of small quantities of material, if the experimental conditions obtaining for the spot reactions of the test and comparison solutions are very similar. In particular, the influences of the accompanying materials, because of the capillary actions of filter paper, affect the aspect of the spot picture much more than they do when spot reactions are carried out on non-porous media. The capillary separation of co-solutes and the local accumulation of reaction products in definite zones of the filter paper are a distinct advantage in qualitative spot reactions on paper, and they may contribute considerably to increasing the sensitivity of the procedure. For quantitative purposes, however, these are a disadvantage. This is easily understood because the production of separate colored zones and rings deprives spot pictures, to a large extent, of their uniformity. The intensities of such pictures are far more difficult to compare than are homogeneous colorations or turbidities in a drop of liquid, or homogeneous colorations on a surface. Therefore, spot colorimetric

determinations based on the union of drops of the test and reagent solutions or on the placing of a drop of solution on reagent paper are sure to be inaccurate if an undisturbed capillary spreading is allowed to occur. The newer method, discussed in the next paragraph, has overcome this defect of the older procedure by a simple device.

The formation of capillary pictures that interfere with spot colorimetric determinations and which are affected seriously by even indifferent accompanying materials, can be avoided if the uninhibited capillary spreading of drops of solution placed on filter paper is prevented. This is accomplished by laying off a reaction field on filter paper that has been impregnated with certain reagents. The boundaries are established by treating the paper with materials that repel water or are impermeable to it. If a drop of solution is placed on this reaction field, there is only a limited possibility for lateral migration; most of the liquid or the materials dissolved in it are forced to pass down into the paper, especially if a penetration in this direction is hastened by applying weak suction. A reaction can then occur with the reagent present in the capillaries of the paper. The result is a constant deposition of colored reaction products and consequently the development of a uniformly colored fleck, which is precisely what is needed for a comparison of colors.

Colorimetric determinations by confinement of the reaction flecks are based, therefore, on the principle that drops of liquid pass through a laterally bounded reagent barrier and thereby deposit colored reaction products and make possible a colorimetric comparison. It is obvious that this principle need not be restricted to single drops, but if insoluble colored compounds are produced, several drops or definite volumes of the liquid can be subjected to this passage. The accuracy of spot colorimetric determinations is thus increased. Comparisons within wider limits of the quantities of materials to be determined can also be made under these circumstances. The following description supplements this discussion.

1. *Confined Spot Test Papers*

The test papers are squares of filter paper, 40 mm. on the side (S. and S., Nos. 211 and 598). The center of each square is impregnated with a ring of a chemically inert material (paraffin, wax, bakelite). This uniform barrier encloses an area of 100 mm.^{2}, 50 mm.^{2}, or 10 mm.^{2}, as shown in Fig. 44.

The choice of the particular reaction field for the spot colorimetric determinations that will be described here, is dictated by the quantity of material to be determined and by the volume of the liquid that is to be passed through the reagent barrier.

The circular boundary will prevent the outward flow of water, aqueous salt

solutions, dilute acids and alkalies, alcohols and other volatile organic liquids, such as acetone, chloroform, etc. Paper No. 211 is absorbent, has a close texture, is free from surface irregularities, and is recommended for all spot tests when the reaction is made with a single drop of solution. No. 598 confined spot test paper is retentive and highly absorbent; its greatest reaction field permits the formation of uniformly colored spots from test portions measuring 5 to 10 ml., thereby extending the confined spot test technic to the analysis of samples of appreciable volume.

The confined spot test papers can be prepared as follows: Very thin tissue paper is dipped in molten paraffin at about 100°C. The excess paraffin is drained off. The paraffined tissue paper, cooled to room temperature, is laid on the filter paper to be impregnated, and is pressed down for a moment, with a brass ring heated to about 90°C. The diameter of

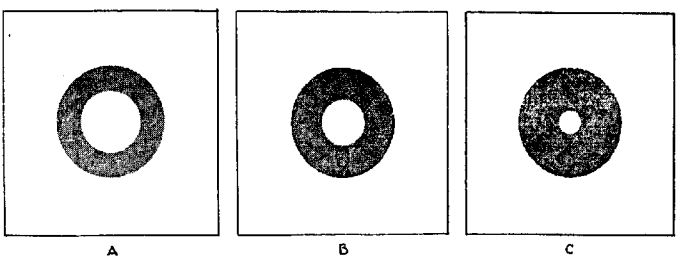


Fig. 44. Confined spot test papers. The paraffin barrier, between the concentric circles, surrounds the reaction area

the rings is 11.3 mm., 8 mm. and 3.6 mm., respectively, corresponding to the reaction fields specified. The warm metal is held with forceps; it melts the paraffin, which is then taken up by the underlying filter paper. When the tissue paper is removed, a paraffin ring is left on the filter paper. The impregnation is made within this bounded area.

Papers provided with paraffin rings prepared by this method are on the market.

2. *Impregnation of the Reaction Field*

In general, it is better to impregnate the reaction field of the filter papers, prepared as described in (1), with the necessary reagents just prior to making the test. It is not well to store prepared reaction papers designed for spot colorimetric determinations, because in many instances the original uniform and fine dispersion of the reagents is lost in the course of time.

Because of recrystallization the reagent becomes loosened from the capillaries of the paper and it dusts off.

The simple apparatus shown in Fig. 45 is suited for charging the reaction field with the necessary reagent. It will also be used in the actual spot colorimetric determinations. The apparatus consists of a wide neck 200 ml. bottle. It is fitted with a two hole stopper carrying a funnel about 3.5 cm. across, and a glass tube bent at right angles. The filter paper, provided with the paraffin ring, is laid on the funnel (C) and the junction made tight by means of the metal ring (B). The reagent solution is brought onto the reaction field in single drops, each drop allowed to evaporate in turn, and this addition is continued until the desired quantity of reagent has been applied. With volatile solutions (alcohol, ether, etc.) the solvent can be removed more quickly if gentle suction is applied through the tube (D).

The best reagents for the procedure described here are those which give definite tests through the formation of intensely colored precipitates. The reagent itself should be colorless, easily soluble in volatile organic solvents, and difficultly soluble in water. If the reagent has these desirable solubility characteristics, a uniform and stable distribution both of the reagent and of the reaction product over the surface of the fleck is almost certainly assured.

Water-soluble reagents can also be used to impregnate a confined reaction surface. A single drop of a concentrated solution is used and the spot is dried at 50° to 60°C. When the reagent is not soluble in alcohol, the water can be removed by adding drops of alcohol.

The reagent must always be used in large excess. A spot surface of 100 mm.^{2} should contain 1 milligram of reagent for each 20 γ of the metal to be precipitated. The quantity of the reagent added is easily computed from the volume and the concentration of the reagent solution. Methyl alcohol has two advantages over ethyl alcohol as a solvent for reagents. The majority of reagents are more soluble in methyl alcohol, and consequently, a smaller number of drops of the saturated reagent solution is required to transfer a given quantity of the reagent onto the reaction field. Furthermore, because of the greater volatility of this solvent, the drops of the reagent solution evaporate faster. Both factors aid in shortening the time necessary to prepare the reaction surface.

It is best to prepare only small quantities of the reagent solutions. They should be kept in readiness in brown glass dropping bottles (15 ml. capacity). The volume of the drops need be determined only once for each solution. The operator then will know the number of drops necessary to bring one milligram of the solid reagent onto the reaction surface. It is well to note the volume of the drop on the label of the bottle.

3. *Procedure for Spot Reactions with Test and Comparison Solutions*

The general rule that the spot reactions with the test and comparison solution should be made as far as possible under the same conditions, holds also when confined reaction surfaces are used. The procedure given here requires that the drops of solution brought onto the reaction surface react immediately with the reagent that it meets there. Consequently, preparative measures, such as adjustment to a definite pH, oxidation, reduction, etc., must be taken beforehand with both the test and comparison solutions.

After the paper has been prepared for the test, as described in 1 and 2, it is placed on the funnel of the apparatus (Fig. 45), the junction made tight with the metal ring, and a definite quantity of the solution (test or comparison) is brought onto the reaction surface. The necessary drops of solution

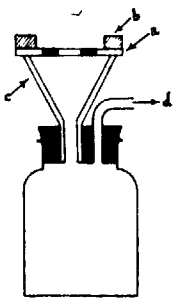


Fig. 45. Apparatus for carrying out quantitative spot tests: (a) paper; (b) metal ring to hold the paper down; (c) funnel; (d) outlet for oral suction (through a rubber tube)

are delivered from pipettes or microburettes, whose tips are drawn down to a fine capillary. The volume of the solution must be in accord with the area of the spotting surface. Volumes of liquid (single or several drops) of 0.1 ml., 0.05 ml., and 0.01 ml. correspond to reaction fields whose areas are 100 mm.^{2}, 50 mm.^{2} and 10 mm.^{2}, respectively. As soon as the precipitation ceases, slight suction is applied (by mouth or pump) through the tube (D) until all the liquid has been collected as drops on the under side of the paper. The hanging drop is taken off with a strip of filter paper, and the rest of the liquid is removed by laying the spot paper on dry filter paper. Finally, the paper is dried at 60° to 90°C. in an oven. In case warming is not permissible, the water retained in the paper can be removed by washing with alcohol; the latter is then allowed to evaporate at room temperature. Fig. 46 is a schematic presentation of the different steps of the procedure. If

several drops are to be added in succession, it is always necessary to wait until the previous drop has been completely absorbed or has run through, before the next drop is brought onto the paper.

If a water-soluble reaction product is formed on the reaction field, it is obvious that only one drop of the test or comparison solution can be added in spot colorimetric determinations. As to the drying of water-soluble reaction products, it should be noted that in the absence of materials that cause decomposition of the paper, the reaction mixture can be evaporated to dryness by placing the test papers in a drying oven heated to 60° to 70°C. The colored compound, along with the other salts present in the original solution, is thus deposited in the fibers of the paper. Ordinarily this procedure does not result in the formation of uniformly colored spots because the dissolved solids tend to migrate toward the periphery of the barrier during the slow evaporation of the water. Advantage can be taken

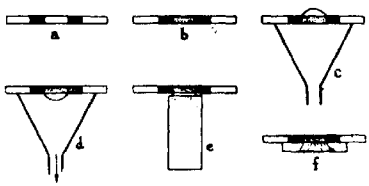


Fig. 46. Stages of a confined spot test which leads to the formation of a colored, insoluble end-product: (a) paper with a water-repellant boundary; (b) paper after evaporation of the alcoholic reagent solution; (c) correct position of a test drop (0.01 ml.); (d) hanging drop after applying suction; (e) removal of the hanging drop; (f) removal of the excess liquid

of this when testing very dilute solutions, because the resulting concentration of the colored substance along the inner boundary of the barrier makes the presence of the constituent being tested far more evident.

4. *Comparison of the Color Intensity*

The flecks produced from test and standard solutions by the foregoing method can be compared in incident or transmitted light. Deeply colored spots are compared most accurately by transmitted light. Spots formed by reaction with a small amount of the element and where the colored precipitate is deposited almost entirely on the upper surface of the test paper can be matched in reflected light. Immersion of the test paper in a clarifying medium such as carbon tetrachloride facilitates the comparison of deeply colored spots in a moderately intense light. This method is particularly useful when the colored compound has penetrated the greater depth of the paper.

Flecks may be compared while they are still moist; this obviates the necessity of drying them. However, in general, it is preferable to compare dried flecks. In no case is it permissible to compare moist and dried flecks because the diverse translucence always causes great differences in the aspect of the spot picture.

It is expedient to prepare a series of standard tests. These are flecks, of a definite reaction product of the material being sought, arranged in the order of decreasing quantity. Such comparison series can be kept on hand, if the flecks are coated with paraffin and thus protected against the action of the atmosphere. For this purpose the strips carrying the dried flecks are immersed for five minutes in a solution of hard paraffin (10 per cent in carbon tetrachloride). After draining and evaporation of the solvent, the whole paper will be coated with a thin film of paraffin. Should the precipitate on the paper be soluble in carbon tetrachloride, the specimen can be preserved by merely dipping the paper into melted paraffin.

The comparison test papers, arranged in the order of the decreasing quantities of the material sought, can be mounted advantageously, like diapositives (lantern slides), between thin glass plates or sheets of cellophane. When not in use, it is well to protect the series of comparison specimens against the action of light by wrapping them in black paper. If paraffined preparations are used for comparison, the fleck of the test solution obviously must also be paraffined after it has been dried. Permanent preparations of the determinations are thus obtained, and in many cases, this is desirable. The colors of paraffined flecks are invariably compared in transmitted light. It is possible to paraffin flecks of soluble colored reaction products; of course, after they have been dried.

D. Practice Exercises

The determinations to be described necessitate no preliminary separations, provided the material to be determined is the main constituent of the specimen. If this metal, however, is present only in small quantities as part of a complicated mixture, preparatory separations must usually be made. The classic methods of separation can be used. Microchemical procedures are recommended if small quantities of the sample are taken. The following discussions include only those details pertinent to the quantitative performance of spot testing.

1. *Aluminum.* The test is carried out on ashless filter paper (S. and S., No. 598 G or No. 211). The reagent is prepared by dissolving 0.01 g. of alizarin in 50 ml. of ethyl alcohol, adding 10 per cent ammonia water, and then diluting with water to 100 ml. A drop (0.05 ml.) is placed on a reaction surface (100 $mm.^2$) and immediately treated with 0.1 ml. of the test solution. Uniform distribution of the dye is best obtained if a current of

air is directed against the surface of the drop through a pipette. The mixture is made alkaline by adding a drop of 0.5 per cent ammonia water. After one or two minutes, the liquid is drawn through the filter paper and removed by the method described previously. A blank is carried through the same procedure. The specimen and the blank are then dried in the air until the original red-violet of the blank has changed to the pale yellow of free alizarin. The specimen is then impregnated with paraffin and is ready to be matched.

2. *Bismuth.* The reagent consists of a saturated solution of cinchonine in methyl alcohol containing 2 per cent of potassium iodide. A small quantity of the reagent is prepared by warming a mixture of 500 mg. of potassium iodide and 300 mg. of cinchonine with 25 ml. of methyl alcohol until completely dissolved. The solution is allowed to stand overnight and the crystals which have separated are then filtered off. Two drops of this solution are enough to impregnate a 100 mm.^{2} reaction fleck. The orange-red color produced by bismuth appears only in acid solution. Accordingly, after the test drop has been removed, the reaction surface is treated with a drop of 1 per cent nitric acid.

3. *Cobalt.* The reaction surface is prepared with one drop of a 5 per cent solution of benzimidazole in methyl alcohol. The purple precipitate, characteristic of cobalt, is developed by adding 0.5 per cent ammonia to the test drop. The alkalization by ammonia can be signaled by means of a thread of red litmus silk placed in the drop.

4. *Nickel.* The reaction surface is impregnated with one drop of a 2 per cent solution of dimethylglyoxime in methyl alcohol. One drop of an ammoniacal nickel solution is placed on the field and the characteristic red color of nickel dimethylglyoxime appears.

5. *Copper.* The surface is prepared with a drop of a 5 per cent solution of benzoinoxime in methyl alcohol. The procedure is like that described for cobalt. Usually the green precipitate is obtained in a flocculent condition. It can be uniformly distributed over the reaction surface if, before drawing the drop into the paper, a stream of air is directed gently against the reaction mixture by means of a pipette.

6. *Iron.* A reaction field (100 mm.^{2}) on ashless paper (S. and S., No. 598 G) is moistened with 0.05 ml. of a freshly prepared 0.02 per cent water solution of potassium ferrocyanide. Without drying the paper, 0.1 ml. of the acidified test solution is brought on the reaction surface. Since the Prussian blue remains essentially in colloidal suspension, the water cannot be removed by suction. Consequently, the whole reaction mixture is evaporated on the spotted surface at 50° to 60°C., or the paper may be allowed to dry by standing at room temperature.

This method permits the determination of iron in solutions that contain

10 to 250 γ Fe per milliliter. Smaller quantities of iron (0.5 to 10 γ Fe per milliliter) can be determined in the same way with the aid of 0.1 per cent ferrocyanide solution and a spotting surface of 50 mm.[2] The use of the more dilute reagent is advisable because of the danger that the color of the Prussian blue may be rendered indistinct by the yellow of the excess ferrocyanide.

The procedure described for Prussian blue is also suitable for the determination of divalent iron by means of α,α'-dipyridyl. A 2 per cent solution in methyl alcohol is used as the reagent.

7. *Silver.* The reaction surface is prepared with a drop of a saturated ethyl alcohol or acetone solution of *p*-dimethylamino-benzylidene rhodanine. A drop of the neutral test solution and a drop of 1 per cent nitric acid are then brought on the surface. After the red precipitate forms, the excess liquid is sucked off and the fleck washed with acetone.

The procedure can be used only for the determination of very small quantities of silver. Because of the slight solubility of the reagent, one drop of the saturated solution is sufficient only for the precipitation of 2 γ silver at the most.

APPENDIX

Auxiliary Reagents

Concentrated Acids

	Specific Gravity	Per cent by Weight	Approximately
Acetic acid, glacial	1.05	99.5	17 *N*
Hydrochloric acid	1.19	37.9	12 *N*
Nitric acid	1.42	69.8	16 *N*
Perchloric acid	1.53	60	9 *N*
Phosphoric acid	1.7	85	15 *N*
Sulfuric acid	1.84	96.0	36 *N*

Diluted Acids

Acetic acid.—Dilute 285 ml. of the concentrated acid to 1 liter with water	5 *N*
Hydrochloric acid.—Dilute 430 ml. of the concentrated acid to 1 liter with water	5 *N*
Nitric acid.—Dilute 310 ml. of the concentrated acid to 1 liter with water	5 *N*
Sulfuric acid.—Pour 140 ml. of the concentrated acid slowly and with constant stirring into 500 ml. of water, cool, and dilute to 1 liter	5 *N*
Sulfurous acid.—Prepare a saturated solution in water	0.3 *N*

Bases

Ammonium hydroxide solution, concentrated.—The commercial product sp. gr. 0.88, contains about 28 per cent NH_3	15 *N*
Ammonium hydroxide solution, dilute.—Dilute 335 ml. of the concentrated solution to 1 liter with water	5 *N*
Barium hydroxide solution.—Shake 70 g. of crystallized barium hydroxide $Ba(OH)_2 \cdot 8H_2O$ with 1 liter of water; filter or siphon off the liquid (saturated solution) and protect from atmospheric CO_2	0.4 *N*
Calcium hydroxide solution.—Shake 2 to 3 g. of calcium hydroxide with 1 liter of water; filter or siphon off the liquid (saturated solution) and protect from atmospheric CO_2	0.04 *N*
Potassium hydroxide solution.—Dissolve 220 g. of the ordinary "pure" sticks (about 90 per cent KOH) in water and dilute to 1 liter	5 *N*
Sodium hydroxide solution.—Dissolve 220 g. of the ordinary "pure" sticks (about 90 per cent NaOH) in water and dilute to 1 liter	5 *N*

Salt Solutions*

Ammonium acetate, $NH_4C_2H_3O_2$ (M.W.77).—Dissolve 231 g. of the salt in 1 liter of water	3 *N*

* The common reagents chlorine water, bromine water, iodine solution, and hydrogen sulfide water are included here.

SALT SOLUTIONS*—*Continued*

	Approximately
Ammonium carbonate (the commercial salt is a mixture of NH_4HCO_3 and $NH_4CO_2NH_2$).—Dissolve 160 g. of the salt in a mixture of 140 ml. of conc. ammonium hydroxide solution and 860 ml. of water	4 *N*
Ammonium chloride, NH_4Cl (M.W.53.5).—Dissolve 270 g. of the salt in 1 liter of water	3 *N*
Ammonium oxalate, $(NH_4)_2C_2O_4 \cdot H_2O$ (M.W.142).—Dissolve 35 g. of the crystalline salt in 1 liter of water	0.5 *N*
Ammonium sulfate, $(NH_4)_2SO_4$ (M.W.132).—Dissolve 132 g. of the salt in 1 liter of water	2 *N*
Yellow ammonium sulfide solution, $(NH_4)_2S_x$.—Use the solution available commercially. It may be prepared, if desired, as follows: Saturate 200 ml. of conc. ammonia solution with H_2S, keeping the solution cold; add 10 grams of flowers of sulfur and 200 ml. of conc. ammonia solution, shake until the sulfur has dissolved, and dilute to 1 liter	6 *N*
Colorless ammonium sulfide solution, $(NH_4)_2S$.—Saturate 2 parts by volume of conc. ammonia solution with H_2S, keeping the solution cold; add an equal volume of conc. ammonia solution and dilute with 6 parts of water. This solution is prepared as required	6 *N*
Barium chloride, $BaCl_2 \cdot 2H_2O$ (M.W.244).—Dissolve 61 g. of the salt in 1 liter of water	0.5 *N*
Bromine water, Br_2 (M.W.160).—A saturated aqueous solution is prepared by shaking 35 grams or 11 ml. of liquid bromine with water	
Calcium chloride, $CaCl_2 \cdot 6H_2O$ (M.W.219).—Dissolve 55 g. of the hydrated salt in 1 liter of water	0.5 *N*
Calcium sulfate, $CaSO_4 \cdot 2H_2O$ (M.W.172).—Shake 3 g. of the salt with 1 liter of water; filter or decant the saturated solution after several hours	0.03 *N*
Chlorine water, Cl_2 (M.W.71).—Saturate 250 ml. of water with chlorine. The chlorine may be generated by dropping conc. HCl upon $KMnO_4$. Preserve in a dark-colored bottle. The solution contains 6.5 grams of Cl_2 per liter	
Cobalt nitrate, $Co(NO_3)_2 \cdot 6H_2O$ (M.W.291).—Dissolve 44 g. of the salt in 1 liter of water	0.3 *N*
Copper sulfate, $CuSO_4 \cdot 5H_2O$ (M.W.249.5).—Dissolve 125 g. of the salt in 1 liter of water containing 10 ml. of dilute sulfuric acid	0.5 *N* (as oxidant)
Ferric chloride, $FeCl_3 \cdot 6H_2O$ (M.W.270).—Dissolve 135 g. of the hydrated salt in 1 liter of water containing 20 ml. of conc. HCl	0.5 *N* (as oxidant)
Ferrous sulfate, $FeSO_4 \cdot 7H_2O$ (M.W.277).—Dissolve 140 g. of the salt in 1 liter of water containing 7 ml. of conc. H_2SO_4	0.5 *N* (as reductant)

SALT SOLUTIONS*—*Continued*

	Approximately
Hydrogen sulfide solution, H_2S (M.W.34).—Saturate 250 ml. of water with H_2S gas. The solution contains approximately 4.2 g. of H_2S per liter	0.05 *N*
Iodine solution, I_2 (M.W.254).—Dissolve 12.7 g. of iodine in a solution of 20 g. of pure KI in 30 ml. of water, and dilute to 1 liter with water	0.1 *N*
Lead acetate, $Pb(C_2H_3O_2)_2 \cdot 3H_2O$ (M.W.379).—Dissolve 95 g. of the salt in 1 liter of water	0.5 *N*
Magnesium sulfate, $MgSO_4 \cdot 7H_2O$ (M.W.246).—Dissolve 62 g. of the salt in 1 liter of water	0.5 *N*
Mercuric chloride, $HgCl_2$ (M.W.272).—Dissolve 27 g. of the salt in 1 liter of water	0.2 *N*
Potassium chromate, K_2CrO_4 (M.W.194).—Dissolve 49 g. of the salt in 1 liter of water	0.5 *N* (as precipitant)
Potassium cyanide, KCN (M.W.65).—Dissolve 32.5 g. of the salt in 1 liter of water	0.5 *N*
Potassium ferricyanide, $K_3Fe(CN)_6$ (M.W.329).—Dissolve 55 g. of the salt in 1 liter of water	0.5 *N* (as precipitant)
Potassium ferrocyanide, $K_4Fe(CN)_6 \cdot 3H_2O$ (M.W.422).—Dissolve 53 g. of the salt in 1 liter of water*	0.5 *N* (as precipitant)
Potassium iodide, KI (M.W.166).—Dissolve 83 g. of the salt in 1 liter of water	0.5 *N*
Potassium permanganate, $KMnO_4$ (M.W.316).—Dissolve 3.2 g. of the salt in 1 liter of water; filter through glass wool or asbestos.	0.1 *N* (as oxidant)
Potassium thiocyanate, KCNS (M.W.97).—Dissolve 49 g. of the salt in 1 liter of water	0.5 *N*
Silver nitrate, $AgNO_3$ (M.W.170).—Dissolve 17 g. of the salt in 1 liter of water	0.1 *N*
Silver sulfate, Ag_2SO_4 (M.W.312).—Dissolve 8 g. of the salt in 1 liter of water. This is nearly a saturated solution	0.05 *N*
Sodium acetate, $NaC_2H_3O_2 \cdot 3H_2O$ (M.W.136).—Dissolve 408 g. of the crystallized salt in 1 liter of water	3 *N*
Sodium carbonate, $Na_2CO_3 \cdot 10H_2O$ (M.W.286).—Dissolve 430 g. of the crystallized salt in 1 liter of water	3 *N*
Disodium hydrogen phosphate, $Na_2HPO_4 \cdot 12H_2O$ (M.W.358).—Dissolve 120 g. of the salt in 1 liter of water	1 *N*
Stannous chloride, $SnCl_2 \cdot 2H_2O$ (M.W.226).—Dissolve 56 g. of the salt in 100 ml. of conc. HCl and dilute to 1 liter. Keep a few pieces of tin in the bottle to prevent oxidation	0.5 *N*

* The reagent used to remove calcium is 2 *N*.

Solid Reagents

Aluminum (turnings or foil), Al
Ammonium chloride, NH_4Cl
Ammonium nitrate, NH_4NO_3
Ammonium thiocyanate, NH_4CNS
Asbestos fiber, for filters
Borax, $Na_2B_4O_7 \cdot 10\ H_2O$
Calcium chloride, $CaCl_2$
Calcium fluoride, CaF_2
Copper (foil or turnings), Cu
Devarda's alloy (powder)
Ferrous sulfate, $FeSO_4 \cdot 7\ H_2O$
Fusion mixture, $Na_2CO_3 + K_2CO_3$
Glass wool
Iron (wire), Fe
Lead dioxide (Mn-free), PbO_2
Manganese dioxide (precipitated), MnO_2
Microcosmic salt $NaNH_4HPO_4 \cdot 4\ H_2O$
Potassium bisulfate, $KHSO_4$
Potassium carbonate, K_2CO_3
Potassium chlorate, $KClO_3$
Potassium cyanide, KCN
Potassium dichromate, $K_2Cr_2O_7$
Potassium nitrate, KNO_3
Potassium nitrite, KNO_2
Potassium iodide, KI
Potassium permanganate, $KMnO_4$
Potassium persulfate, $K_2S_2O_8$
Silica, SiO_2
Sodium acetate (fused), $NaC_2H_3O_2$
Sodium bicarbonate, $NaHCO_3$
Sodium bitartrate, $NaHC_4H_4O_6$
Sodium hydroxide, NaOH
Sodium nitrite, $NaNO_2$
Sodium nitroprusside, $Na_2[Fe(CN)_5NO] \cdot 2\ H_2O$
Sodium sulfite, $Na_2SO_3 \cdot 7\ H_2O$
Stannic chloride, $SnCl_4 \cdot 5\ H_2O$
Stannous chloride, $SnCl_2 \cdot 2\ H_2O$
Starch (soluble)
Sulfur (flowers), S
Tartaric acid, $H_2C_4H_4O_6$
Tin (pure foil), Sn
Zinc (20 mesh), Zn

Standard Solutions Containing 50 γ Cation per Drop (0.05 ml.)

Cation	*Formula of Dry Compound*	*Grams of Compound per Liter of Solution*	*Special Precaution in Preparation of the Solution*
Al^{+++}	$Al_2(SO_4)_3 \cdot 18\ H_2O$	12.35	Use 2 ml. of dilute H_2SO_4
	or $K_2SO_4 \cdot Al_2(SO_4)_3 \cdot 24\ H_2O$	17.59	Use 2 ml. of dilute H_2SO_4
Ag^{+}	$AgNO_3$	1.58	
As^{+++} (ous)	As_2O_3	1.32	Dissolve in hot water containing 2 ml. of dilute HCl and dilute to 1 liter
As^{+++++} (ic)	As_2O_5	1.53	As for arsenious solution
	or $Na_2HAsO_4 \cdot 12\ H_2O$	5.37	
Au^{+++}	$AuCl_3 \cdot 2\ H_2O$	1.72	
Ba^{++}	$Ba(NO_3)_2$	1.90	
Be^{++}	$Be(NO_3)_2 \cdot 4\ H_2O$	22.74	
Bi^{+++}	$Bi(NO_3)_3 \cdot 5\ H_2O$	2.32	Dissolve salt in 10 ml. of dilute HNO_3 and dilute to 1 liter
Ca^{++}	$Ca(NO_3)_2 \cdot 4\ H_2O$	5.89	
Cd^{++}	$Cd(NO_3)_2 \cdot 4\ H_2O$	2.78	Use 0.5 ml. of dilute HNO_3
Ce^{+++}	$Ce(NO_3)_3 \cdot 6\ H_2O$	3.03	
Co^{++}	$Co(NO_3)_2 \cdot 6\ H_2O$	4.94	
Cr^{+++}	$CrCl_3 \cdot 6\ H_2O$	5.12	Use 5 ml. of dilute HCl
	or $K_2SO_4 \cdot Cr_2(SO_4)_3 \cdot 24\ H_2O$	9.60	Use 5 ml. of dilute H_2SO_4

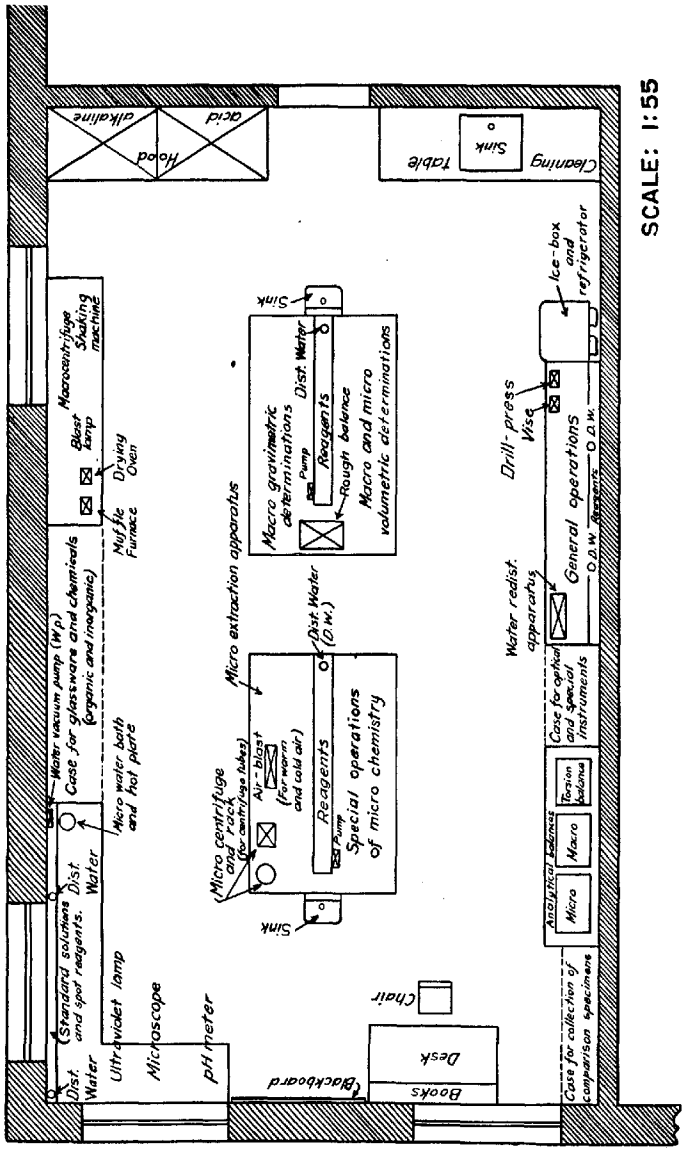
STANDARD SOLUTIONS CONTAINING 50 γ CATION PER DROP (0.05 ML.)—*Continued*

Cation	*Formula of Dry Compound*	*Grams of Salt per Liter of Solution*	*Special Precaution in Preparation of the Solution*
Cs^{+}	$CsNO_3$	1.47	
Cu^{++}	$CuSO_4 \cdot 5\ H_2O$	3.93	Use 0.5 ml. of dilute H_2SO_4
Fe (ous)	$FeSO_4 \cdot (NH_4)_2SO_4 \cdot 6\ H_2O$	7.03	Use 2 ml. of dilute H_2SO_4
Fe (ic)	$FeCl_3 \cdot 6\ H_2O$	4.84	Use 5 ml. of dilute HCl
Hg^{+} (ous)	$Hg_2(NO_3)_2 \cdot 2\ H_2O$	1.40	Use 10 ml. of dilute HNO_3
Hg^{++} (ic)	$HgCl_2$	1.35	
K^{+}	KCl	1.91	
	or KNO_3	2.59	
Li^{+}	$LiNO_3$	9.94	
Mg^{++}	$Mg(NO_3)_2 \cdot 6\ H_2O$	10.54	
Mn^{++}	$MnSO_4 \cdot 4\ H_2O$	4.07	
	or $MnCl_2 \cdot 4\ H_2O$	3.60	
Na^{+}	NaCl	2.54	
	or $NaNO_3$	3.70	
NH_4^{+}	NH_4Cl	2.97	
	or NH_4NO_3	4.44	
Ni^{++}	$NiSO_4 \cdot 7\ H_2O$	4.78	
	or $NiCl_2 \cdot 6\ H_2O$	4.05	
Pb^{++}	$Pb(NO_3)_2$	1.60	Use 0.5 ml. of dilute HNO_3
Pd^{++}	$Pd(NO_3)_2$	2.16	
Pt^{++++}	$PtCl_4$	1.73	
Sb^{+++} (ous)	$SbCl_3$	1.88	Dissolve in 50 ml. of dilute HCl and dilute to 1 liter
Sb^{+++++} (ic)	$SbCl_5$	2.45	As for antimonous solution
Sn^{++} (ous)	$SnCl_2 \cdot 2\ H_2O$	1.90	Dissolve in 10 ml. of conc. HCl and dilute to 1 liter
Sn^{++++} (ic)	$SnCl_4 \cdot 5\ H_2O$	2.95	Dissolve in 50 ml. of dilute HCl and dilute to 1 liter
Sr^{++}	$SrCl_2 \cdot 6\ H_2O$	3.04	
	or $Sr(NO_3)_2$	2.42	
Ti^{+++}	$Ti_2(SO_4)_3$	4.01	Add dilute H_2SO_4 to obtain a clear solution
Tl^{+}	$TlNO_3$	1.30	
U^{++++++}	$UO_2(NO_3)_2 \cdot 6\ H_2O$	2.11	
Zn^{++}	$ZnSO_4 \cdot 7\ H_2O$	4.40	
	or $Zn(NO_3)_2$	2.90	
Zr^{++++}	$Zr(OH)_4$	1.75	Dissolve in dilute HNO_3 and dilute to 1 liter

STANDARD SOLUTIONS CONTAINING 50 γ CATION PER DROP (0.05 ML.)—*Continued*

Anion	*Formula of Dry Compound*	*Grams of Salt per Liter of Solution*
CO_3^{--}	$Na_2CO_3 \cdot 10\ H_2O$	4.93
SO_3^{--}	$Na_2SO_3 \cdot 7\ H_2O$*	3.15
$S_2O_3^{--}$	$Na_2S_2O_3 \cdot 5\ H_2O$*	2.22
S^{--}	$Na_2S \cdot 9\ H_2O$*	7.51
SeO_3^{--}	SeO_2	0.87
TeO_3^{--}	K_2TeO_3	1.22
NO_2^-	$NaNO_2$*	1.50
CN^-	KCN	2.50
CNO^-	KCNO	1.93
CNS^-	KCNS	1.69
$Fe(CN)_6^{----}$	$K_4Fe(CN)_6 \cdot 3\ H_2O$	1.99
$Fe(CN)_6^{---}$	$K_3Fe(CN)_6$	1.55
Cl^-	NaCl	1.65
Br^-	KBr	1.49
I^-	KI	1.31
F^-	NaF	2.21
NO_3^-	$NaNO_3$	1.37
ClO_3^-	$KClO_3$	1.47
BrO_3^-	$KBrO_3$	1.31
IO_3^-	KIO_3	1.22
ClO_4^-	$KClO_4$	1.41
IO_4^-	KIO_4	1.21
BO_3^- (Borate)	$Na_2B_4O_7 \cdot 10\ H_2O$	2.25
SO_4^{--}	$Na_2SO_4 \cdot 10\ H_2O$	3.36
$S_2O_8^{--}$	$K_2S_2O_8$	1.42
SiO_3^{--}	Na_2SiO_3	1.61
SiF_6^{--} (Silicofluoride)	Na_2SiF_6	1.32
PO_4^{---}	$Na_2HPO_4 \cdot 12\ H_2O$	3.77
HPO_3^{--} (Phosphite)	$Na_2HPO_3 \cdot 2\ H_2O$	2.70
$H_2PO_2^-$ (Hypophosphite)	$NaH_2PO_2 \cdot H_2O$	1.63
N_3^- (Azide)	NaN_3	1.55
CrO_4^{--}	K_2CrO_4	1.67
$Cr_2O_7^{--}$	$K_2Cr_2O_7$	1.36
MnO_4^-	$KMnO_4$	1.33
VO_3^-	KVO_3	1.40
MoO_4^{--}	$(NH_4)_6Mo_7O_{24} \cdot 4\ H_2O$	1.23
WO_4^{--}	$Na_2WO_4 \cdot 2\ H_2O$	1.33
$C_2H_3O_2^-$ (Acetate)	$NaC_2H_3O_2 \cdot 3\ H_2O$	2.31
$H \cdot CO_2^-$ (Formate)	$H \cdot CO_2Na$	1.35
$C_2O_4^{--}$ (Oxalate)	$Na_2C_2O_4$	1.52
$C_4H_4O_6^{--}$ (Tartrate)	$NaKC_4H_4O_6 \cdot 4\ H_2O$	1.91
$C_6H_5O_7^{---}$ (Citrate)	$Na_3C_6H_5O_7 \cdot 2\ H_2O$	1.56
$C_7H_5O_3^-$ (Salicylate)	$C_6H_4(OH)CO_2Na$	1.17
$C_7H_5O_2^-$ (Benzoate	$C_6H_5CO_2K$	1.32
$C_4H_4O_4^{--}$ (Succinate)	$C_2H_4(CO_2Na)_2$	1.40

* These solutions do not keep well and should be freshly prepared.



Floor Plan of a Spot Test Laboratory

INDEX